PHYLOGENETIC RELATIONSHIPS OF THE *OXYTROPIS CAMPESTRIS* AND *OXYTROPIS ARCTICA* COMPLEXES IN ALASKA INFERRED FROM NON-CODING NUCLEAR DNA AND RAPD DATA

BY

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A

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ABSTRACT

The taxonomy and evolutionary relationships of the *Oxytropis arctica* and *Oxytropis campestris* complexes in Alaska are poorly understood. Taxonomic disagreement has centered on which morphological characters are important in circumscribing these taxa. Several of these taxa are endemic to Alaska, including *Oxytropis arctica* var. *barnebyana*, which is currently of conservation concern. Internal transcribed spacer sequences and randomly amplified polymorphic DNA markers were employed to circumscribe these taxa. Both lines of evidence revealed one major dichotomy dividing northern populations from western populations.

There is weak support for traditional taxonomies. Morphological characters used to separate these taxa do not assort to either side of the dichotomy. These traits may be controlled by one or a few genes and may not represent degrees of divergence. They may have been derived independently in each population in response to adaptation to local environmental conditions, changing quickly in response to natural selection, genetic drift, mutation, or migration.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

The genus *Oxytropis* belongs to the cosmopolitan pea family Fabaceae, which is comprised of 643 genera representing 18,000 species (Mabberly 1997). In Alaska, *Oxytropis* is a relatively large and complex genus consisting of about 23 taxa. Several taxa found in interior and arctic Alaska are derived from two major polyploid complexes, *O. arctica* and *O. campestris*, and include both endemic as well as widespread species (Figure 1).

Taxonomic consensus on these complexes has long been lacking. In which complex do certain taxa belong, and what taxonomic rank should they be given? Various qualitative and quantitative characters have traditionally been used to discriminate among these taxa, and wide variation of the morphological characters has been reported (Appendix 1). According to Welsh (1991), in the genus *Oxytropis* there are few morphological characters that can be used as taxonomic criteria, and the large number of synonyms is a direct corollary of the problematic nature of the taxa in this genus (Table 1). Although boundaries between these taxa can be blurred, there are core morphological traits that have been used to circumscribe the *O. arctica* and *O. campestris* complexes. Differences such as flower color (blue vs. white), number of flowers per inflorescence (few vs. many), habit (erect /tall vs. procumbent /dwarf), and flower size (small vs. large) have predominantly been used. Nevertheless, extensive debate exists over which of these characters deserves the most weight, or whether they should even be weighted.

The importance of flower color as a diagnostic character for these taxa appears to be at the center of this debate. For example, whereas the *O. arctica* complex consists largely of blue-flowered plants and the *O. campestris* complex consists of nearly white or pale yellow ones, plants from these two complexes are difficult to distinguish when they are in fruit. The taxonomy proposed by Welsh (1968) placed the yellow-flowered narrow endemic taxon (*O. arctica* var. *barnebyana*) within the otherwise blue-flowered *O. arctica* complex (which includes the amphi-Beringian *O. arctica* var. *arctica* and the endemic *O. arctica* var. *koyukukensis*) based on larger flower size. Welsh (1974) also included the predominantly blue-flowered (*O. roaldi* Ostenf.) within the *O. arctica* complex, although he recently annotated specimens from the Vascular Plant Herbarium (DAO) in Ottawa, Canada as belonging within the *O. campestris* complex (Cody 2000). This complex traditionally includes both the restricted taxon *O. campestris* ssp. *jordalii* and the widespread taxon *O. campestris* ssp. *gracilis* . Welsh appears to emphasize flower size (smaller in *O. campestris* vs. larger in *O. arctica*) over flower color.

Conversely, according to the Russian botanist, B. A. Yurtsev (1999), "American authors evidently underestimate the diagnostic value of colour of petals." Yurtsev has proposed an alternative taxonomy in which the white/cream flowered var. *barnebyana* is treated as most closely related to the Eurasian yellow-flowered *O. sordida* which has been considered as belonging to the *O. campestris* complex (Barneby 1952). Several other taxa are morphologically similar to the *O. arctica* and

O. campestris complexes but are not considered part of these complexes. For example, the blue-flowered, narrow endemic *O. kobukensis*, according to Welsh (1967), has affinities to *O. arctica.* The recently named Alaskan endemic *O. tananensis* Jurtz. is morphologically similar to members of the *O. campestris* complex (Yurtsev 1999). Beyond flower color and size, these complexes do not appear to be definitively distinguished. It is questionable whether there are indeed consistent morphological traits that can be relied upon to distinguish these taxa. If not, it is reasonable to ask whether they really are distinct biologically based taxa. The answer has practical applications.

Oxytropis arctica var. *barnebyana* is listed in the Alaska Rare Plant Field guide (Lipkin and Murray 1997). It holds a global rank assigned by The Nature Conservancy and an international network of Natural Heritage Programs and Conservation Data Centers of G4T2, indicating that it is endangered throughout its range. It holds a state rank of S2, meaning imperiled in the state of Alaska because of rarity (6-20 occurrences), or because of other factors making it very vulnerable to extirpation from the state). It is also listed as rare by the Conservation of Arctic Flora and Fauna Program (CAFF) (Talbot et al. 1999). In 1996 a conservation agreement between the U.S. Fish and Wildlife Service and the U.S. Air Force was signed to cooperatively conserve populations of *O. arctica.* var. *barnebyana* occurring in the Kotzebue, Alaska vicinity (Moran 1997). This agreement was in response to the

impending closure of the Air Force facilities and subsequent demolition of asbestos-containing buildings in the area.

The aim of this study was to resolve phylogenetic relationships and test the congruence of molecular data with the current morphologically based taxonomies in the *O. arctica* and *O. campestris* complexes. Currently, there are no published studies of phylogenetic relationships among species of *Oxytropis* in Alaska, and to date research in this area has consisted largely of alpha taxonomy.

The main questions I am addressing are:

1. Do multi-locus molecular data corroborate the taxonomic entities of the *O. campestris* and *O. arctica* complexes?
2. Are single and apparently simple diagnostic characters (for example, corolla color blue vs. white, number of flowers per inflorescence < 5 vs. > 5, or flower size < 15 mm or >15 mm) congruent with the relationships exhibited by molecular data?
3. Is there a geographic component to the relationships revealed by molecular characters?

Several different regions of the plant genome have provided characters useful for phylogenetic reconstruction, yet the choice of marker for a study depends on the level of variation under study (for review of markers see Soltis and Soltis (1998)). Chloroplast (cpDNA) sequence data, in which the variation is fairly highly conserved, have been widely used for phylogenetic reconstruction at higher taxonomic levels. The

mitochondrial genome has not often been used for systematic studies at lower taxonomic levels due to the high sequence conservation typical of plants. In addition, plant mt DNAs undergo intermolecular recombination resulting in heteroplasmy (Palmer 1992). I selected the Internal Transcribed Spacer (ITS) region of the nuclear ribosomal DNA because it has proven useful for phylogenetic reconstructions at lower taxonomic levels, for example, in *Saxifraga* sect. *saxifraga* (Vargas 2000), *Fragaria* (Potter and Harrison 2000), *Armeria* (Aguilar et al. 1999), and *Astragalus* (Wojciechowski 1993). ITS comprises two non-coding regions (ITS1 and ITS2) of the nuclear ribosomal DNA separated by the 5.8S ribosomal gene and flanked by the 18S and 26S ribosomal genes (Figure 2). It contributes an ideal source of DNA sequence variation for the following reasons: i) ITS 1 and ITS 2 are under less stringent constraints than the actual coding regions (18S, 5.8S and 26S), thus they have a faster rate of nucleotide substitution; ii) the flanking genes (18S and 26S) provide highly conserved priming sites, thus expediting *in vitro* amplification (Baldwin et al. 1995). Concerted evolution of the ITS region usually ensures that only orthologous rather than paralogous DNA sequences are compared, although examples of a lack of concerted evolution have been reported (e.g., see Buckler et al. 1997).

Because phylogenetic reconstruction at low taxonomic levels among angiosperms cannot be based on the cytochrome b or the control region of mt DNA, (both have been widely used for population level studies of vertebrates and other animals), non-sequencing techniques such as Inter Simple Sequence Repeats (ISSR),

Amplified Fragment Length Polymorphisms (AFLP) and Random Amplified Polymorphic DNA (RAPD) have been used for capturing useful genetic information among closely related species. The RAPD technique has been widely used in taxonomic studies ranging from intergeneric to single population studies (Wolfe and Liston 2000). I chose the RAPD method as a means for producing markers (or bands) generated by arbitrary primers, which can detect polymorphisms potentially from all over the genome (Welsch and McClelland 1990; Williams et al. 1990). This "fingerprinting technique" is a PCR-based assay, which can be used for analyzing genetic relatedness among and within species.

Although the RAPD technique has been used for taxonomic studies and conservation studies (Aares et al. 2000, Rossetto et al. 1995), it is not without limitations. Inferences about population structure and nucleotide divergence based on RAPD markers have been considered dubious by many. Perez et al. (1998) stated that reproducibility of results is highly dependent on the quality and concentration of the DNA, the specific polymerase used, and the assay conditions. In addition to high levels of subjectivity in band scoring, co-migrating, anonymous fragments may be non-homologous. For example, amplification of RAPD bands may represent paralogous loci of homoeologs in polyploids (Jessup 1993). Homology can be tested via Southern hybridization or by using restriction enzymes. Several factors can contribute to deviations from Mendelian inheritance using the RAPD method. Besides the technical limitations, scoring of codominant loci and/or heteroduplex bands can

also affect estimates of relatedness. In addition, estimates of genetic diversity may not meet the requirements of Mendelian inheritance if scored markers originate from organellar genomes that are uniparentally inherited (Wolfe and Liston 1998).

MATERIALS AND METHODS

Plant Identification

All voucher specimens were identified using the currently available and widely used treatments listed in Appendix 1. Morphology, ecology, geographic location, and comparisons with authenticated previous collections were considered. Plants from the No Name and North Fork populations along the Squirrel River were identified as *O. arctica* var. *barnebyana* by S. L. Welsh (Moran and Meyers 1996).

Materials

Leaf material was collected from various locations (Figure 3 and Table 2) and was stored either in silica gel, liquid nitrogen, or as fresh frozen leaf tissue. Voucher specimens were collected for each population and deposited in the University of Alaska Herbarium in Fairbanks, Alaska (Appendix 2). Two to four leaflets were collected per individual, and leaf tissue for up to 30 individuals was collected per population. Twenty-six individuals sampled from 17 populations representing 9 taxonomic entities were assessed for phylogenetic relationships using ITS.

O. nigrescens (Pall.) Fisch. subsp. *bryophila* (Greene) Hulten, also referred to as *O. gorodkovii* was selected as the outgroup. *Oxytropis nigrescens* belongs in

section *Arctobia*, whereas *O. campestris* and *O. arctica* belong in the section *Orobia* (Yurtsev 1999) with the exception of *O. tananensis* Jurtz., which belongs in the section *Baicalia*, although it is similar to the *O. campestris* complex (Yurtsev 1993). The section status for *O. kobukensis* is not specified, but according to Welsh (1967), it is most closely related to *O. arctica*, and was, therefore, not used as an outgroup. Outgroup taxa are necessary in parsimony analyses to determine the direction of character evolution (Maddison et al. 1984).

For RAPD analyses, 16 populations were sampled for a total of 89 accessions representing six taxonomic entities (Table 3): *O. arctica* var. *arctica* is represented by 17 individuals from five populations, *O. arctica* var. *barnebyana* is represented by 40 individuals from five populations, *O. arctica* var. *koyukukensis* is represented by 14 individuals from two populations, *O. campestris* var. *gracilis* is represented by 8 individuals from two populations, *O. campestris* var. *jordalii* is represented by 5 individuals from one population, and *O. tananenis* is represented by 5 individuals from one population.

DNA Isolation

DNA was extracted from samples using the CTAB protocol of Doyle and Doyle (1987), as modified by Soltis et al. (1991). All individuals used for RAPD analyses were extracted using DNeasy Plant Mini kits (QIAGEN Inc., Valencia, CA).

ITS PCR and Cycle-Sequencing

Amplifications and sequencing reactions were performed using a Perkin Elemer (2400 series) thermal cycler. ITS PCR amplifications were performed in 50 µl reactions using 10-20 ng of genomic DNA, 3 mM $MgCl_2$, Perkin Elmer 10X PCR buffer containing 15 mM $MgCl_2$, 0.2 µM each dNTP, 1.25 units (5U/µl) Perkin Elmer AmpliTaq Gold DNA polymerase, and 1 mM of each amplification primer. The Internal Transcribed Spacer region was amplified using primers ITS5 (5'-GGAAGGAGAAGTCGTAACAAGG-3') and C26A (5'-GTTTCTTTTCGTCCGCT-3') (Yokota et al. 1989). The PCR protocol included: an initial nine minutes at 95 °C; three cycles of 30 seconds denaturing (94 °C), 1 min. annealing (55 °C), and 45 seconds extension (72 °C); 3 cycles of 30 seconds denaturing (94 °C), 1 min. annealing (53 °C) and 45 seconds extension (72 °C); 20 cycles of 30 seconds denaturing (94 °C), 30 seconds annealing (51 °C), and 45 seconds extension (72 °C); with a final 10 minute elongation at (72 °C), the product was then held indefinitely at 4 °C.

PCR products were separated on a 1% agarose gel with ethidium bromide run at 90 V for 40 minutes. Gels were visualized under UV light. Amplification products were cleaned using a QIAquick PCR Purification Kit (QIAGEN Inc., Chatsworth, CA) following the protocol from the manufacturer. Bands were rechecked on a 1% agarose gel to insure that the PCR product remained present after cleanup.

Sequencing reactions were performed using primers: ITS1F (5'-TCCGTAGGTGAACCTGCGG-3'), ITS2R (5'-GCTRCGTTCTTCATCGATAC-3'), ITS3F (5'-GCATCGATGAAGAACGYAGC-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990; Baldwin 1992) in 10 µl reactions. Each reaction included: 4 µl sequenase (Dye Terminator Cycle Sequence Ready Reaction with AmpliTaq DNA, Perkin Elmer), 5.0 µl PCR template, and 1.0 µl of 3.2 pm/µl primer. The sequencing cycle included: an initial denaturation for 4 min. (80 °C), 25 cycles of 10 seconds denaturing (96 °C), 5 seconds annealing (50 °C), and 4 min. extension (60 °C), with a final 10 minutes at 4 °C, and held at 4 °C indefinitely. Samples were sequenced using dye fluorescence terminator chemistry on a Perkin Elmer 373 Stretch XL automated sequencer.

ITS Sequence Alignment and Phylogenetic Analyses

Sequences were aligned manually. Phylogenetic analyses were performed with PAUP*4.0b2, (Swofford 1999) using Maximum Parsimony (Swofford et al. 1996), Neighbor-joining (Saitou and Nei 1987), and Maximum Likelihood (Felsenstein 1981) methods.

With gaps coded as missing data, heuristic searches were replicated 100 times using random stepwise addition sequences, tree bisection-reconnection (TBR) branch swapping, and MULTREES option in effect. Statistical support of lineages was estimated by bootstrapping (Felsenstein 1985). Bootstrapping is a resampling technique that generates a number of pseudo data sets where the columns are

randomly drawn and replaced a number of times, and then each pseudo data set is analyzed with parsimony and the most parsimonious tree deriving from each pseudo-replicate is calculated. Bootstraps were done using a fast-heuristic search, and stepwise addition with 100 random addition sequences.

The software MODELTEST (Posada and Crandall 1998) was used to evaluate likelihood models. The maximum likelihood algorithm uses a number of evolutionary models to evaluate the net likelihood of an observed sequence substitution.

RAPD DNA Quantification, PCR, Primer Selection and Band Scoring

DNA was quantified via visual inspection by running the DNA out on a 1% agarose gel and comparing band intensities to a known concentration of standard size marker (λ cut with a double digest of HindIII and EcoRI). Additionally, DNA was quantified using a fluorometer and diluted to appropriate concentrations. Various DNA concentrations (from 0.01 ng to 2.0 ng) were tested to determine the best reliable PCR products.

Primers were selected from kits A, C, and D (Operon Technologies, Alameda, CA). Primers useful for generating banding patters for *Oxytropis* were selected from a pre-tested list (Brochmann 1999). A total of 6 primers were determined to be potentially useful for scoring. Primers A-11 (5'-CAATCGCCGT-3'), C-05 (5'-GATGACCGCC-3'), C-15 (5'-GACGGATCAG-3'), D-03 (5'-GTCGCCGTCA-3'), D-07 (5'-TTGGCACGGG-3'), and D-08 (5'-GTGTGCCCCA-3') were used, producing 23 polymorphic bands.

PCR amplifications were performed in a Perkin Elmer GeneAmp PCR System 9600 thermal cycler. The PCR reaction included: an initial 3 minutes at 94 °C; 35 cycles of 15 seconds denaturing (94 °C); 30 seconds annealing (39 °C), 60 seconds extension (72 °C) with a final 5 -minute elongation at (72 °C); and the product was then held indefinitely at 4 °C. To run the RAPD gel, 5 µl of 6 X loading dye was put into each 25 µl reaction. Then, 25 µl of this mix was loaded into each well along with 15 µl λ (cut with HindIII/EcoRI) molecular weight marker on a 1.4% agarose gel. Gels were run for 3 hours. Gels were visualized under UV light at 302 nm. Pictures were taken with an Electrophoresis Systems Photo-Documentation Camera (Fisher Scientific) using Professional Coaterless B &W Instant Pack Film (ISO 3000/36°, processing 30 sec. at 75 °F).

Bands were scored as either present or absent using a conservative approach where only distinct, reliable, and reproducible bands were scored. Gels were repeated to ensure reproducibility.

RAPD Analysis

The software program NTSYS-pc (Rohlf 1998) was used to perform a clustering analysis using the unweighted pair group method with arithmetic averaging (UPGMA). The binary matrix was transformed to similarity measures using the Dice coefficient. Dice's coefficient lends weight to matches rather than mismatches (Sneath and Sokal 1973) and does not take shared absences of bands into account. The UPGMA analysis produces a dendrogram based on overall similarity.

RESULTS

ITS Sequences and Phylogenetic Analyses

Twenty-six individuals representing nine taxa including the outgroup, *O. nigrescens* ssp. *bryophila*, were used in the ITS analyses. Sequence length was constant across the sampled individuals. The total length of the ITS1, 5.8S and ITS2 regions was 599 base pair (bp), including 163 bp of the 5.8S region. Borders of these three regions were established by comparison with sequences of *O. campestris* var. *johannensis* (L.) DC. (Wojciechowski et al. 1993) available from Genbank. Phylogenetic analyses employed 439 nucleotide positions, including 227 bp of the ITS 1 region, 2 bp at the 3' end of the 5.8S gene, 209 bp of the ITS 2 region, and 1 bp at the beginning of the 26 gene (Table 3).

Parsimony analyses resulted in 4,675 most parsimonious trees. Each of the most parsimonious trees had a length of 17 steps, a consistency index (CI) of 0.824, a retention index (RI) of 0.923, and a rescaled consistency index (RC) of 0.760. The relatively high (CI) value indicated a strong phylogenetic signal in the data with only ten parsimony informative characters.

In the parsimony analyses (Fig. 4), two major splits were recognizable, with one major clade in the first split and two clades in the second split. The first split was supported by a bootstrap value of 52% and consisted of four taxa: the yellow-flowered *O. campestris* var. *jordalii* (MTH), and the white-flowered *O. tananensis* (CLI) from Delta (sister taxa supported with 32% bootstrap), the white flowered

population of *O. arctica* var. *barneybana* (VAB) from Prudhoe Bay, and two blue-flowered *O. arctica* var. *arctica* populations, (IBP) from a Prudhoe Bay Pingo, and (SAG) just south of Prudhoe Bay which clustered together with 40% bootstrap support.

The second major split resolved two clades. One clade was supported with a 25% bootstrap value and included three taxa: *O. arctica* var. *barnebyana* from two populations in western Alaska (NOR, NON), *O. arctica* var. *arctica* from the Seward Peninsula (TOR), and three individuals of *O. arctica* var. *koyukukensis* from Wiseman (WIS) which clustered together with 66% bootstrap support. The other clade included five taxa: *O. arctica* var. *barnebyana* from populations in western Alaska and the Seward Peninsula (KOT, NON, KUG), *O. campestris* ssp. *gracilis* from three populations (RIC, DAL, FAI), and *O. kobukensis* (KOB) from western Alaska. This clade was weakly supported by an 18% bootstrap value.

The strict consensus of the most parsimonious trees (not shown) resulted in an unresolved clade of the above taxa which were supported by the 52% bootstrap value. The three accessions of *O. arctica* var. *koyukukensis* from the Wiseman population clustered together. All other accessions were represented in an unresolved polytomy.

The MODELTEST program selected the Jukes Cantor+G substitution model with a –Ln Likelihood = 738.4506, all rates equal, equal frequencies, proportion of invariable sites = 0, and gamma = 0.006. Using the MODELTEST parameters, maximum likelihood analysis resulted in three trees with similar topologies. The

maximum likelihood tree shown in Fig. 5 was generally congruent with the maximum parsimony tree with the exception of one *O. arctica* var. *barnebyana* individual which differed in its relative position within the clades nested in the second major split. Maximum likelihood bootstrap values ranged from 2% to 67%.

The neighbor-joining analysis was also generally congruent with the maximum likelihood and maximum parsimony analyses (Fig. 6). The neighbor-joining tree (using Jukes-Cantor distance and a gamma shape parameter of 0.006) supported the same taxa within the first major split with the following differences: blue-flowered *O. arctica* var. *arctica* from Prudhoe Bay (IBP) Pingo was basal to white-flowered *O. arctica* var. *barnebyana* from Prudhoe VAB population. *O. arctica* var. *barnebyana* (VAB) was basal to *O. arctica* var. *arctica* (SAG) leaving a sister taxa relationship of *O. campestris* ssp. *jordalii* and *O. tananensis* as the most derived taxa. The remaining taxa represented in the second major split remained relatively the same within the two clades.

Sequence divergence among the taxa using Jukes-Cantor distance ranged from 0.00% - 1.48% (Table 4). The greatest divergence was between *O. kobukensis* and *O. tananensis* at 1.48%. Sequence divergence within the *O. arctica* complex ranged from 0.00% to 1.41%. Within *O. arctica* var. *barnebyana* divergence ranged from 0.00% to 0.92%. Within *O. arctica* var. *koyukukensis,* divergence ranged from 0.00% to 0.94%. The divergence range for populations of *O. arctica* var. *arctica* was 0.46% to 1.41%.

Sequence divergence within the *O. campestris* complex ranged from 0.00% to 1.40%. Within populations of *O. campestris* ssp. *gracilis* sequence divergence was 0.00%.

ITS data analyzed by maximum parsimony, maximum likelihood, and neighbor-joining analyses revealed the following: 1) Monophyly of the *O. arctica* complex was not supported. 2) Monophyly of the *O. campestris* complex was not supported. 3) Populations of *O. arctica* var. *arctica* were polyphyletic and were represented in both major splits. 4) Populations of *O. arctica* var. *barnebyana* were polyphyletic and were represented in both major splits. 5) The two populations of *O. koyukukensis* , one from Wiseman (WIS) and the other from Minnie Creek (MIN), did not cluster together, although members of the (WIS) population formed a monophyletic group. 6) Populations of *O. campestris* ssp. *gracilis* were not monophyletic although they were found only in one of the two major splits of the ITS and RAPD trees. 7) *Oxytropis* populations from Prudhoe Bay (IBP, SAG,VAB) in northern Alaska were separated from *Oxytropis* populations from western Alaska (KOT, NOR, NON, KUG, TOR, KOB). Populations south of the Brooks Range were found within both major clades, MTH and CLI within the northern clade; and MIN, RIC, DAL, FAI, and WIS, within the other.

RAPD UPGMA Clustering

Twenty-three polymorphic RAPD markers were scored for 89 individuals (Fig. 7, Table 5). The dendrogram in Fig. 8 shows a topology that is similar to the ITS-

generated cladograms discussed above. The most significant differences between the two lines of evidence were: the change in position of the *O. campestris* ssp. *jordalii* (MTH) population, and the change in position of the Minnie Creek (MIN) population of *O. arctica* var. *koyukukensis.* In the ITS trees, *O. campestris* ssp. *jordalii* was clustered with *O. tananensis* and the three northern populations (VAB, IBP, SAG), but in the RAPD tree this population was positioned with *O. arctica* var. *barnebyana* individuals from western Alaska (cluster I). The Minnie Creek population of *O. arctica* var. *koyukukensis* was clustered with populations from western Alaska in the ITS trees, but was located with populations from northern Alaska (cluster IV) in the RAPD tree. The RAPD tree revealed one major dichotomy. One of the major splits had two clusters. Individuals of *O. tananensis* clustered together (cluster V) and were clearly separated from other taxa. The second cluster (cluster IV) represented within this major split included all three populations from the Prudhoe Bay Arctic Coastal Plain (SAG, IBP and VAB) and one population of *O. arctica* var. *koyukukenis* (MIN) from the Brooks Range.

The second major split (cluster I, II, and III) also supported relationships similar to those revealed by the ITS data, with the exception of *O. campestris* ssp. *jordalii.* The most basal clustering (cluster I) included individuals from four populations of *O. arctica* var. *barnebyana.* (KOT, NON, NOR, KUG), all individuals of *O. campestris* ssp. *jordalii*, and some individuals of *O. campestris* ssp. *gracilis* (RIC, BIR). In a more distal cluster (cluster II), members of the *O. arctica* complex

formed the following clade: all individuals of *O. arctica* var. *arctica* (TOR) from western Alaska, all individuals of *O. arctica* var. *koyukukensis* (WIS) from south of the Brooks Range, and both individuals of *O. arctica* var. *arctica* from northern Canada (PEA, VIC). Three individuals of *O. campestris* ssp. *gracilis* and one individual of *O. arctica* var. *arctica* (IBP) where at the base of the second split (cluster III).

To summarize, RAPD phenotypes analyzed by UPGMA clustering showed the following: 1) Monophyly of the *O. arctica* complex was not supported. 2) Monophyly of the *O. campestris* complex was not supported. 3) Populations of *O. arctica* var. *arctica* were paraphyletic and were represented in both major splits. 4) Populations of *O. arctica* var. *barnebyana* were represented in both major splits. Populations from western Alaska (KOT, NOR, NON, KUG) were found only in the first cluster (I) but were not monophyletic. 5) The two populations of *O. arctica* var. *koyukukensis* were represented in both major splits. 6) Populations of *O. campestris* ssp. *gracilis* were not monophyletic, although they were found only in one of the major splits. 7) *Oxytropis* populations from Prudhoe Bay (IBP, SAG,VAB) and from western Alaska (KOT, NOR, NON, KUG, TOR) each belonged to one of the two major splits in the UPGMA tree and were clearly separated. However, populations located south of the Arctic Coastal Plain were found within both major splits: MIN, and CLI clustered with northern populations (cluster IV and V) and MTH, RIC, DAL, FAI, and WIS, clustered with western populations.

DISCUSSION

Phylogenetic relationships among populations in the *O. arctica* and *O. campestris* complexes do not follow simple patterns. Variation in ITS sequences and RAPD phenotypes show weak biogeographic patterns, weak support for traditional taxonomies, little hierarchical structure, and little or no correspondence with morphological traits. These results are consistent with earlier taxonomic studies that often resulted in contrasting systems of classification.

Cladistic analyses of ITS sequences and cluster analysis based on RAPD phenotypes reveals one major dichotomy indicating two groups of populations with similar genetic characteristics. All three populations from the northern Arctic Coastal Plain (SAG, VAB, IBP), along with the *O. tananensis* (CLI) population from central Alaska, clustered together and make up one group of populations with similar genetic characteristics. Populations from western Alaska (KOT, NON, NOR, KUG, and TOR) always clustered together and made up the second group. *O. kobukensis* (KOB) from the Kobuk Sand Dunes, collected less than 100 km from some of the other western populations, also clustered in the second group. Some of the remaining populations located south of the Arctic Coastal Plain and in central Alaska clustered with the northern populations, while others clustered with the western populations. The correspondence between geographic proximity and genetic similarity is striking considering that it was revealed in two very different lines of evidence.

Although both the ITS and the RAPD trees show a major dichotomy dividing populations from western Alaska from populations in northern Alaska, there was some discordance between data sets. *O. campestris* ssp. *jordalii* (MTH) clustered with the northern populations in the ITS analyses but with the western populations in the RAPD analysis. The *O. arctica* var. *koyukukensis* population from Minnie Creek (MIN) clustered with the western populations in the ITS trees but in the RAPD analysis that population clustered with the northern populations. Incongruent data sets are not unusual and can be explained by several factors (Johnson and Soltis 1998). It is likely that these taxa are of recent origin, and, therefore, insufficient time has elapsed for the ITS region to diverge. Thus, lack of sufficient ITS data may have contributed to the differences in placement of certain taxa in the two data sets.

Morphological traits do not clearly assort between the dichotomy revealed by the molecular data. Populations with blue flowers were found on both sides of the dichotomy. The TOR, WIS, PEA, and VIC populations are all blue-flowered and were in the "western" cluster in the RAPD analysis. The SAG, IBP, and MIN populations also have blue flowers and were located in the "northern" split. In the ITS trees, KOB and WIS are both blue flowered populations found in the "western" split. Yet the IPB and SAG populations are also blue-flowered, and they clustered in the "northern" split. White- or yellow-flowered populations also fail to assort between the dichotomy in both data sets. In the ITS trees KOT, NON, NOR, KUG, DAL, RIC, and FAI populations are white- or yellow-flowered and they clustered on one side of

the dichotomy, whereas MTH, CLI, and VAB are white-flowered populations which clustered together in the other major split of the dichotomy.

The number of flowers per inflorescence being either < 5 or > 5 also fails to correspond with either side of the dichotomy in either data set. In the ITS data set, few-flowered populations (MTH, VAB, IPB, and SAG) cluster along with many-flowered populations (CLI) in the "northern" cluster. Likewise, few-flowered populations (KOT, NON, NOR, KUG, TOR, and KOB) cluster together with many-flowered populations (RIC, DAL, FAI, WIS, and MIN) in the "western" cluster. RAPD analysis showed a similar pattern for this character.

A third diagnostic morphological character, flower size < 15 mm vs. > 15 mm, cannot be mapped exclusively on one side or the other. Large-flowered populations such as (KOT, NON, NOR, KUG, TOR, MIN, WIS, and KOB) are in one split, and other "large-flowered" populations (VAB, IBP, and SAG) are in the other split. In the ITS trees, small-flowered populations (RIC, DAL, FAI) and (MTH, CLI) cluster in the "western" and "northern" splits respectively. Similarly, the RAPD tree also showed small-flowered populations on both sides of the dichotomy. MTH, RIC, and BIR clustered in the "western" split, whereas the small-flowered CLI population clustered in the "northern" split.

The disparities in both molecular and morphological data mirror the lack of consensus among alpha taxonomists. The disagreement by taxonomists over which characters are important to delineate these taxa seems inevitable considering these

results. Yurtsev (1999) emphasized the importance of flower color in defining these complexes. He has annotated white-flowered Prudhoe Bay specimens from the University of Alaska Museum Herbarium as the same taxon (*barneybana*) as the white-flowered specimens from Kotzebue (KOT), yet they are on opposite sides of the dichotomy in both the ITS and RAPD data sets. Conversely, Welsh has put more emphasis on flower size than flower color. In that case, the white-flowered population (VAB) from Prudhoe Bay could conceivably be called *O. arctica* var. *arctica.* If so, populations of *O. arctica* var. *arctica* would cluster nicely in both data sets with one major exception: the one population of *O. arctica* var. *arctica* sampled from the Seward Peninsula (TOR). In all analyses, this population clusters on the opposite side from the other *O. arctica* var. *arctica* populations. The two populations of *O. arctica* var. *koyukukensis* (MIN and WIS) fail to cluster together in either data set. In the RAPD analysis, the Minnie Creek population (MIN) clusters with other members of the *O. arctica* complex in the "northern" split. But again, these populations are on opposite sides of the dichotomy from several other *O. arctica* populations. All populations of *O. arctica* var. *barnebyana* with the exception of the Prudhoe Bay population (VAB) cluster in the "western" split. With the exception of a few accessions of *O. campestirs* ssp. *gracilis*, the *O. arctica* var. *barnebyana* populations cluster with other populations of the *O. campestris* complex. Based on that relationship, a weak argument could be made in favor of placing *O. arctica* var. *barnebyana* within the *O.campestris* complex. Regardless, based on the RAPD data,

populations of *O. arctica* var. *barnebyana* from the "western" split cannot be distinguished genetically from *O. campestris* ssp. *gracilis* or *O. campestris* ssp. *jordalii* . *O. tananensis* clusters with *O. campestris* ssp. *jordalii* in the ITS data. This relationship is supported by Yurtsev's description of *O. tananensis* (Yurtsev 1993) where he alluded to a close relationship between *O. tananensis* and *O. davisii* (*O. jordalii* ssp. *davisii*) of British Columbia (see Elisens and Packerd, 1980). Nevertheless, that relationship is not supported by the RAPD data.

The dichotomy revealed by both molecular data sets could indicate that these groups of populations represent separate lineages. If so, then convergent or rapid morphological evolution could explain the discordance between the molecular data and traditional taxonomies based on morphological traits. Because morphological traits are often subject to natural selection, they can follow a different pattern of evolution than molecular markers, which may have little adaptive significance (Schaal et al. 1998). The taxonomic complexity in these complexes could be caused by use of morphological traits that may be controlled by only one or a few loci. According to Gottlieb (1984), many simple dichotomous morphological traits such as growth habit (erect vs. prostrate), height (tall vs. short), achenes (winged vs. wingless) have been shown in some species to be governed by only one or a few genes. Gottlieb stated that due to the open, less integrative and plastic pattern of morphogenesis in plants, large changes in morphology can be accounted for by relatively few genetic changes. More recently, several molecular genetic studies have also shown that characters such as

determinate vs. indeterminate flowering (Bradley et al. 1997) and flower color and shape (Bradshaw et al. 1995) may result from small genetic differences. Studies by Durbin et al. (2000) have shown that a single mutation in the chalcone synthase genes is responsible for the switch from purple to albino phenotype in *Ipomea* (Solanaceae). On the other hand, if the morphological traits used to separate these taxa are polygenic, differences among populations may represent genetic changes throughout the genome (Coyne and Lande 1985). But, quantitative morphological traits can conceal information that other more taxonomically significant traits might show (Hansen et al. 2000). In these northern *Oxytropis* populations quantitative characters such as flower color, flower size, and number of flowers per inflorescence provide little insight into the phylogenetic relationships within the group, suggesting that they too may evolve quickly in response to factors such as local selection pressure, founder effect, and genetic drift.

The small amount of molecular differentiation observed among these populations suggests that they may have become isolated fairly recently. Many Alaskan plant species survived the last ice age in refugial populations, later to spread across the landscape with the retreat of the ice sheets. Rapid spread into their present locations could explain the low amounts of differences among populations at the molecular level, while factors such as natural selection may have brought about the morphological differences as new range was colonized.

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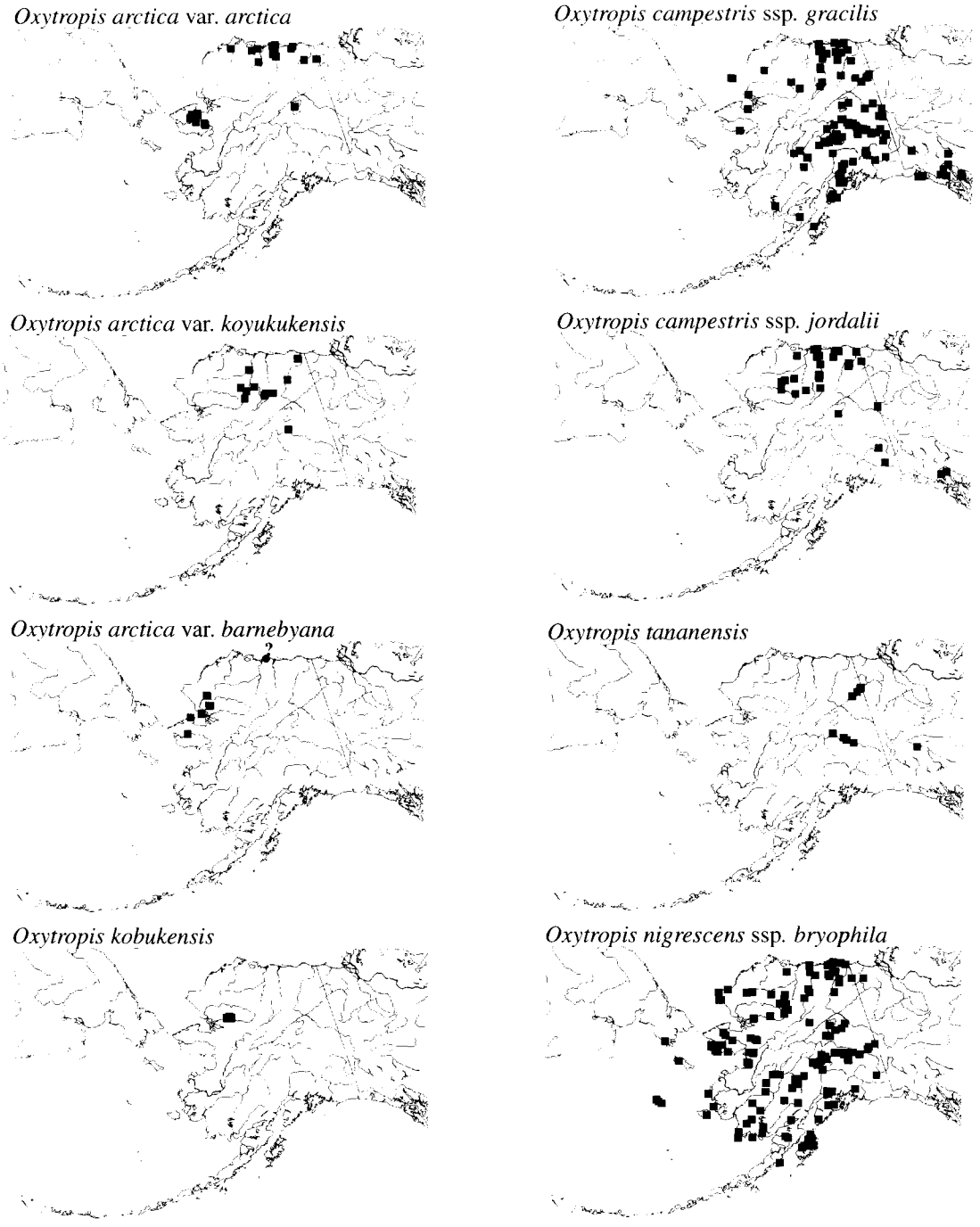


Figure 1. Distribution maps for the *O. arctica* and *O. campestris* complexes and other taxa in this study based on specimens from the University of Alaska Herbarium.

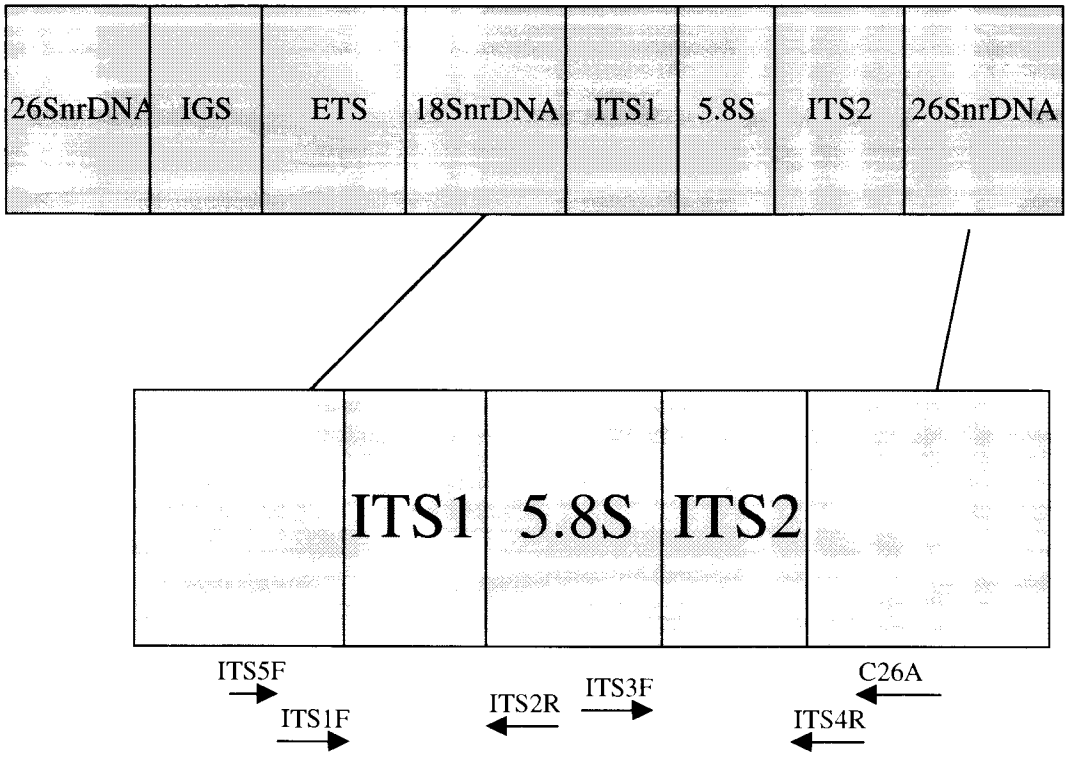
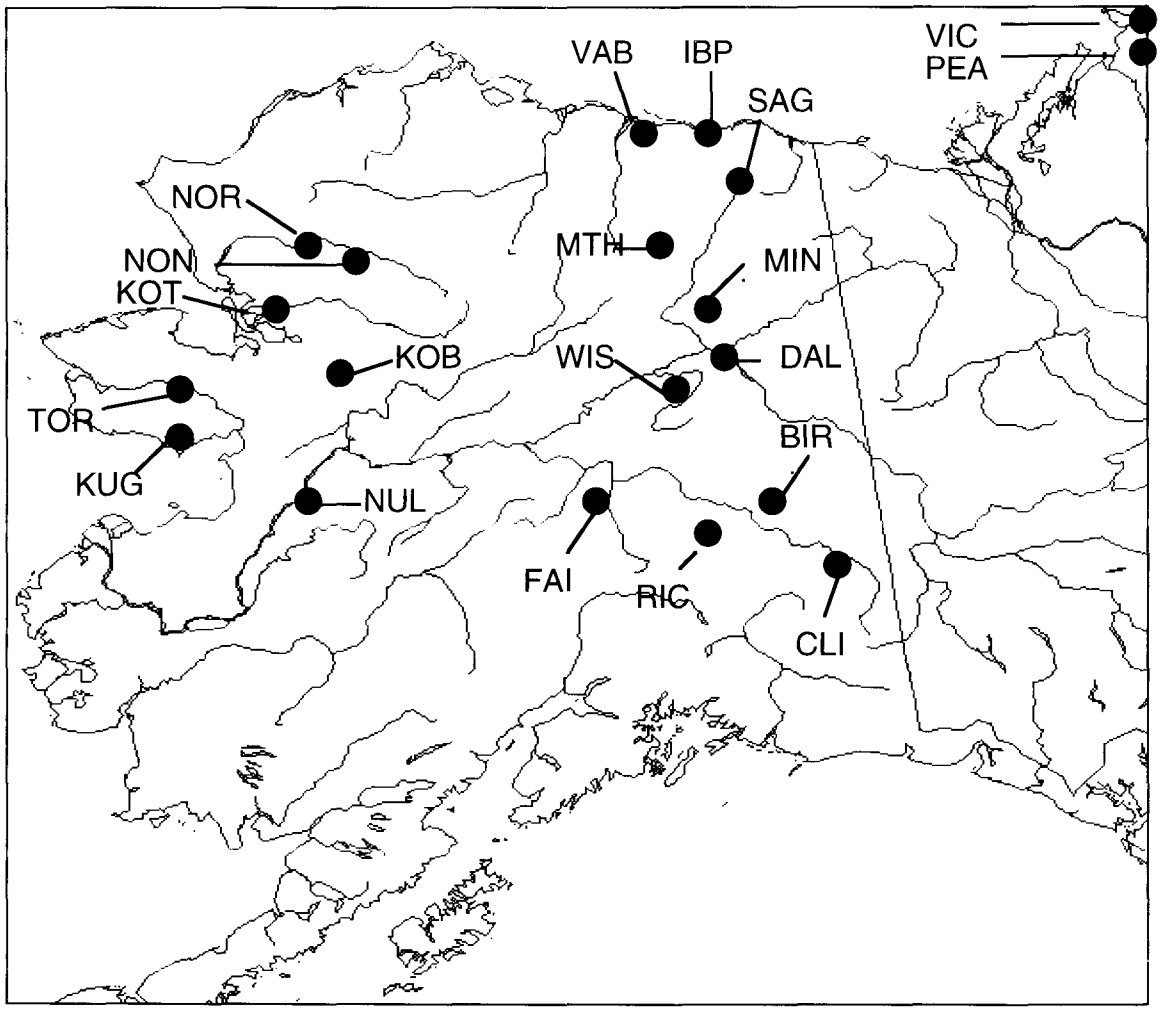


Figure 2. Internal Transcribed Spacer Regions of the nrDNA



Oxytropis arctica var. *barnebyana*	KOT, NOR, NON, KUG, VAB
Oxytropis arctica var. arctica	TOR, IBP, SAG, VIC, PEA
Oxytropis arctica var. *koyukukensis*	WIS, MIN
Oxytropis campestris ssp. *gracilis*	RIC, BIR, DAL, FAI
Oxytropis campestris ssp. *jordalii*	MTH
Oxytropis tananensis	CLI
Oxytropis kobukensis	KOB
Oxytropis nigrescens ssp. *bryophila*	NUL

Figure 3. Collection sites of 20 population of *Oxytropis* in Alaska and Canada.

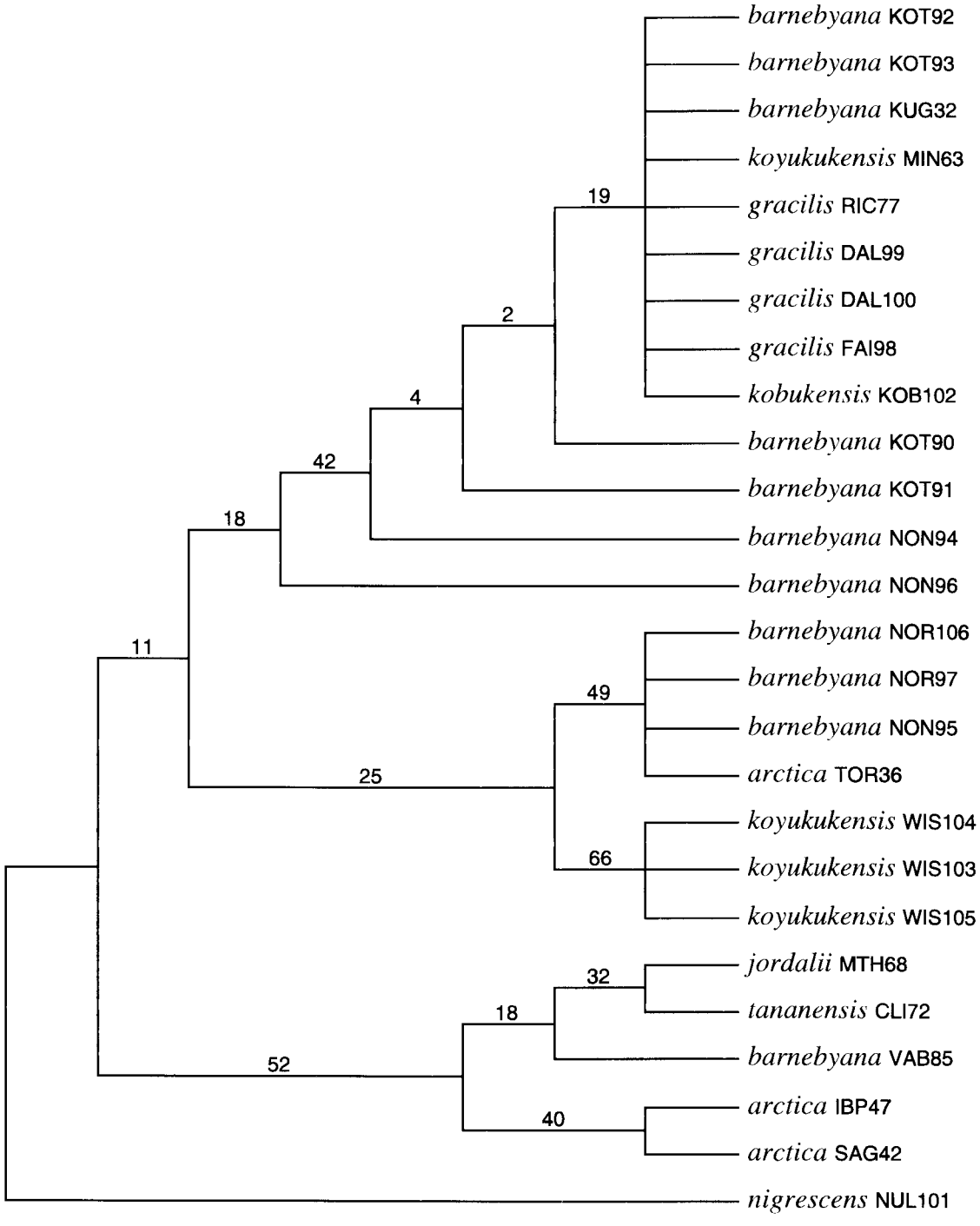


Figure 4. One of 4,675 most-parsimonious trees based on nrDNA internal transcribed spacer sequences of 26 samples of *Oxytropis* (length = 17, CI = 0.824, RI = 0.923 and RC = 0.760). Numbers above branches indicate bootstrap values.

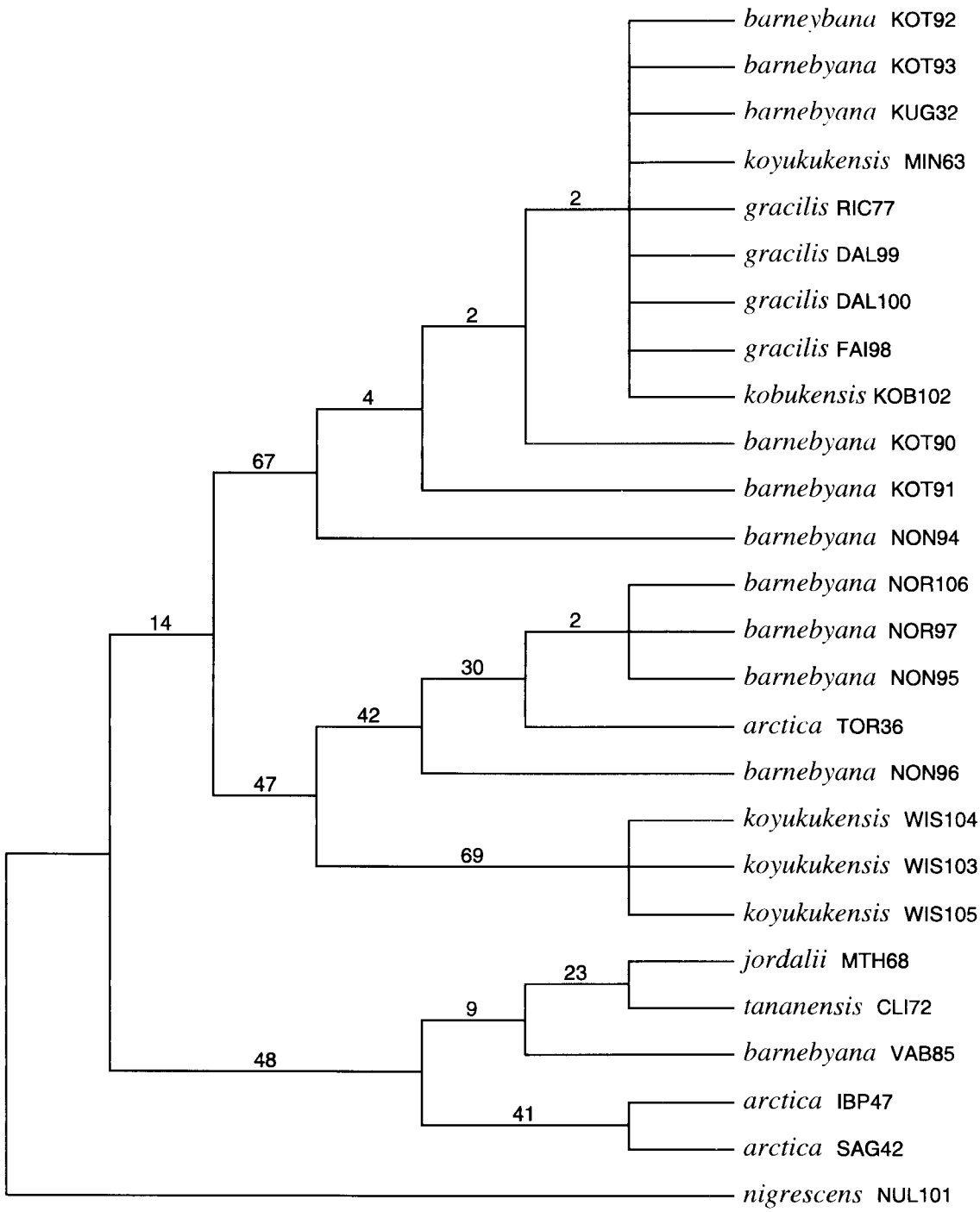


Figure 5. One of three ITS phylogenies based on maximum likelihood analysis (-Ln likelihood = 738.4506). Numbers above branches indicate bootstrap values.

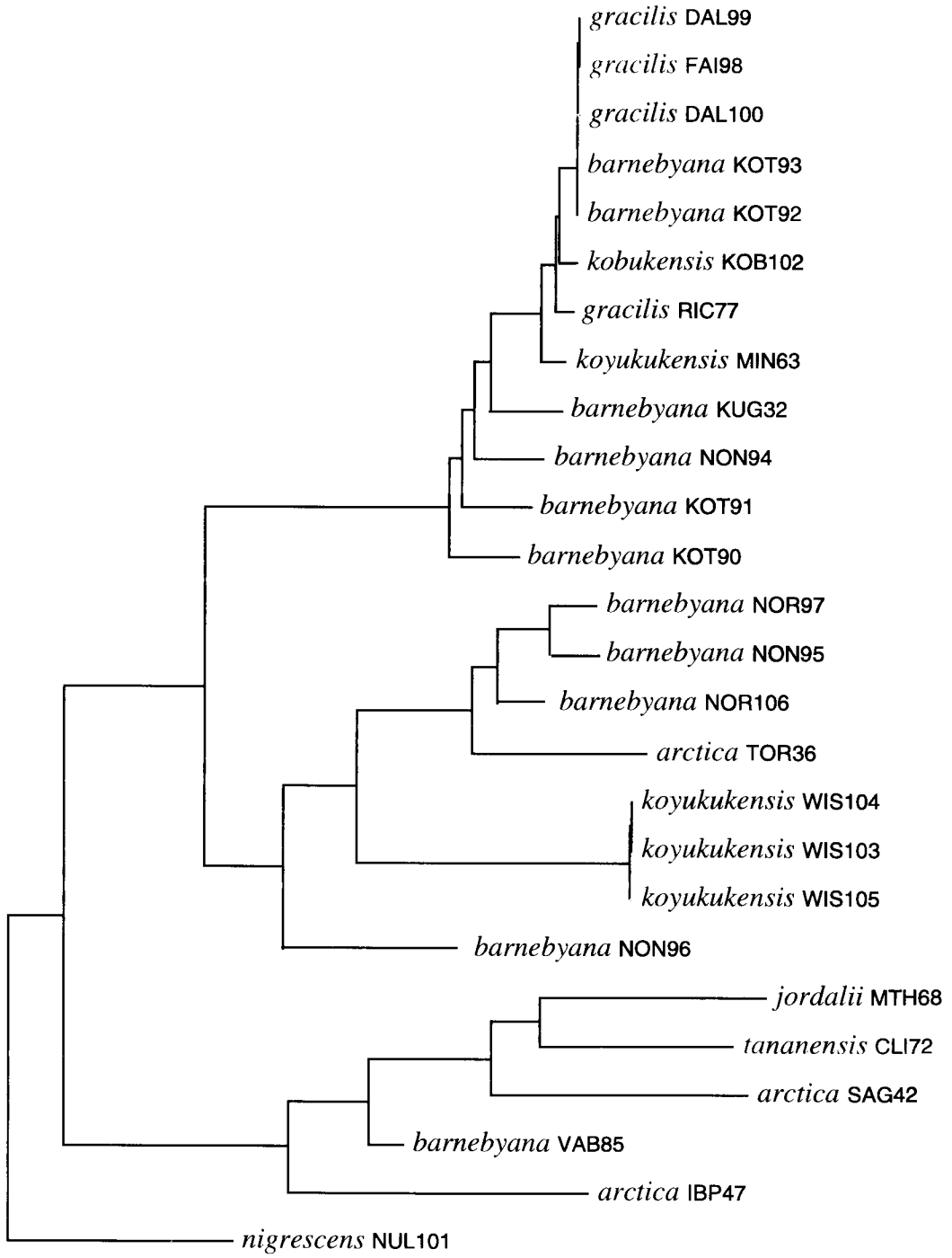


Figure 6. ITS phylogeny resulting from neighbor-joining analysis using Jukes-Cantor distance. The gamma shape parameter was estimated at 0.006.

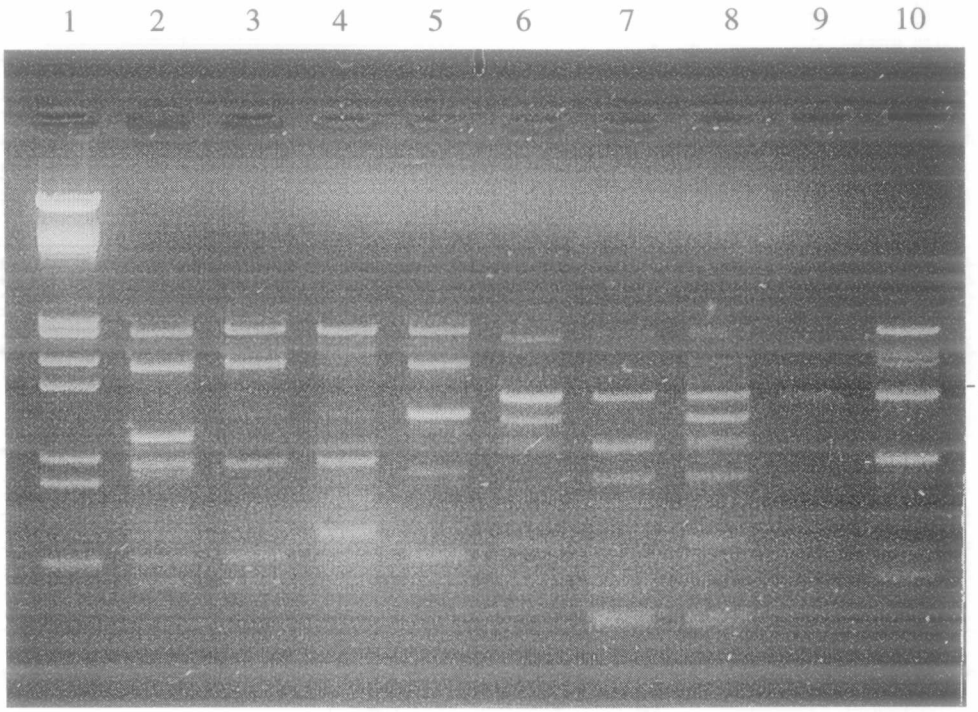


Figure 7. RAPD gel of primer A-11 showing the scored marker at 1300 base pair. Bands were scored as either present = 1 or absent = 0. Sequence of lanes (1-10) on the gel were: λ– marker, CLI 73 = 0, CLI 74 = 0, CLI 75 = 0, CLI 76 = 0, RIC 77 = 1, BIR 78 = 1, BIR 79 = 1, RIC 80 = 1, and RIC 81 = 1.

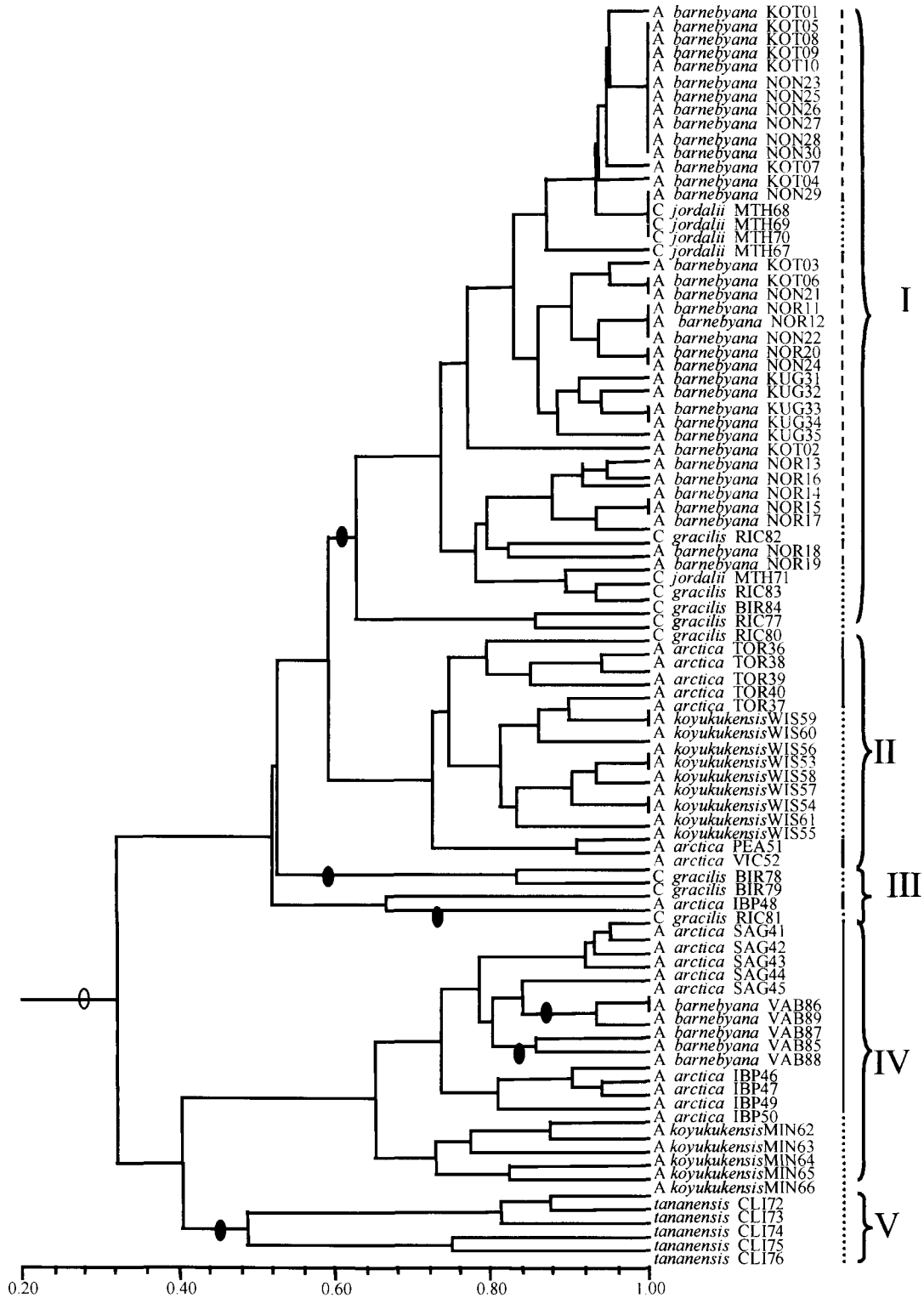


Figure 8. Cluster analysis (UPGMA) of 89 plants from 16 populations. Similarity based on RAPD phenotypes generated by 23 polymorphic markers. Roman numerals identify major clusters. Northern populations indicated by ——— western by - - - and central by ·····. Flower color mapped on the tree as: blue =0 and white/yellow =●.

Table 1. Synonyms for *Oxytropis* taxa.

Oxytropis arctica* R. Br. var. *arctica
O. uralensis of American authors
O. roaldi Ostenfeld, in part
O. coronaminis Fernald.

***Oxytropis arctica* R. Br. var. *barnebyana* Welsh**
O. sordida Willd. subsp. *barnebyana* (Welsh) Yurtsev

***Oxytropis arctica* R. Br. var. *koyukukensis* (Porsild) Welsh**
O. koyukukensis Porsild.
O. roaldi Ostenfeld., in part

***Oxytropis campestris* (L.) DC. subsp. *gracilis* (Nelson) Hulten**
Astragalus campestris L.
Astragallus gracilis Nels.
O. gracilis (Nels.) Schum.
O. campestris var. *gracilis* (Nels.) Barneby
Astragalus varians Rydb.
O. varians (Rydb.) Hult.
O. campestris var.*varians* (Rydb.) Barneby
O. hyperborea Pors.
O. alaskana Nels.

***Oxytropis campestris* (L.) DC. subsp. *jordalii* (Porsild) Hulten**
O. jordalii Pors.
O. campestris var. *jordalii* (Pors.) Welsh

***Oxytropis kobukensis* Welsh**

Oxytropis nigrescens* (Pall.) Fisch. subsp. *bryophila
Astragalus nigrescens Pall.
Astragallus bryophilus Greene
Oxytropis nigrescens var. *bryophila* (Greene) Lepage.

***Oxytropis tananensis* Yurtsev**

Table 2. Summary of samples of *Oxytropis* populations used for ITS and RAPD data. Additional collection data for all samples and voucher specimens are in Appendix 1.

Species/ Pop. #	Location	Lat./Long.	ITS	RAPD
Oxytropis arctica var. *arctica*				
SAG	Sagavanirktok River, Beechey Point Quad	70°01'N 148°38'W	1	5
IBP	IBP Pingo, Prudhoe Bay, Beechey Point Quad	70°16'N 148°34'W	1	5
TOR	Tor near Harris Dome, Seward Peninsula, Bendeleben Quad	65°31'N 164°36'W	1	5
PEA	Pearce Point, Northwest Territories, Canada	69°46'N 122°05'W	0	1
VIC	Victoria Island, Falaise Bay, Nunavut, Canada	69°20'N 114°50'W	0	1
Oxytropis arctica var. *barnebyana*				
KOT	Kotzebue, Baldwin Peninsula, Kotzebue Quad	66°54'N 162°35'W	4	10
NOR	North Fork, Squirrel River, Baird Mountain Quad	67°29'N 161°01'W	2	10
NON	No Name, Squirrel River, Baird Mountain Quad	67°26'N 161°19'W	3	10
KUG	Kougarok Road, Seward Peninsul, Bendeleben Quad	65°23'N 164°40'W	1	5
VAB	VABM Pingo, Prudhoe Bay, Beechey Point Quad	70°28'N 149°43'W	1	5
Oxytropis arctica var. *koyukukensis*				
WIS	Wiseman, Alaska, Wiseman Quad	67°25'N 150°05'W	3	9
MIN	Minnie Creek, Mile 187.2 Dalton Hwy, Wiseman Quad	67°27'N 150°08'W	1	5
Oxytropis campestris ssp. *gracilis*				
RIC	Mile 280 Richardson Hwy, Big Delta Quad	64°13'N 145°58'W	1	5
BIR	Mile 306.5 Richardson Hwy, Birch Lake, Big Delta Quad	64°20'N 146°40'W	0	3
DAL	Dalton Hwy, Marian Cr. Campground, Wiseman Quad	67°19'N 150°09'W	2	0
FAI	Fairbanks, Alaska, Sheep Cr. Road, Fairbanks Quad	64°53'N 147°54'W	1	0
Oxytropis campestris ssp. *jordalii*				
MTH	Mt. Hulten, Dalton Hwy, Philip Smith Quad	68°26'N 149°21'W	1	5
Oxytropis tananensis				
CLI	Cliff, confl. of Delta and Tanana Rv., Big Delta Quad	64°10'N 145°50'W	1	5
Oxytropis kobukensis				
KOB	Great Kobuk Sand Dunes, Ambler River Quad	67°06'N 158°50'W	1	0
Oxytropis nigrescens ssp. *bryophila*				
NUL	Nulato Hills, Debauch Mtn, Norton Bay Quad	64°31'N 159°53'W	1	0

Table 3. Aligned matrix of DNA sequences (5' to 3') of the Internal Transcribed Spacer region including ITS1 and ITS2.

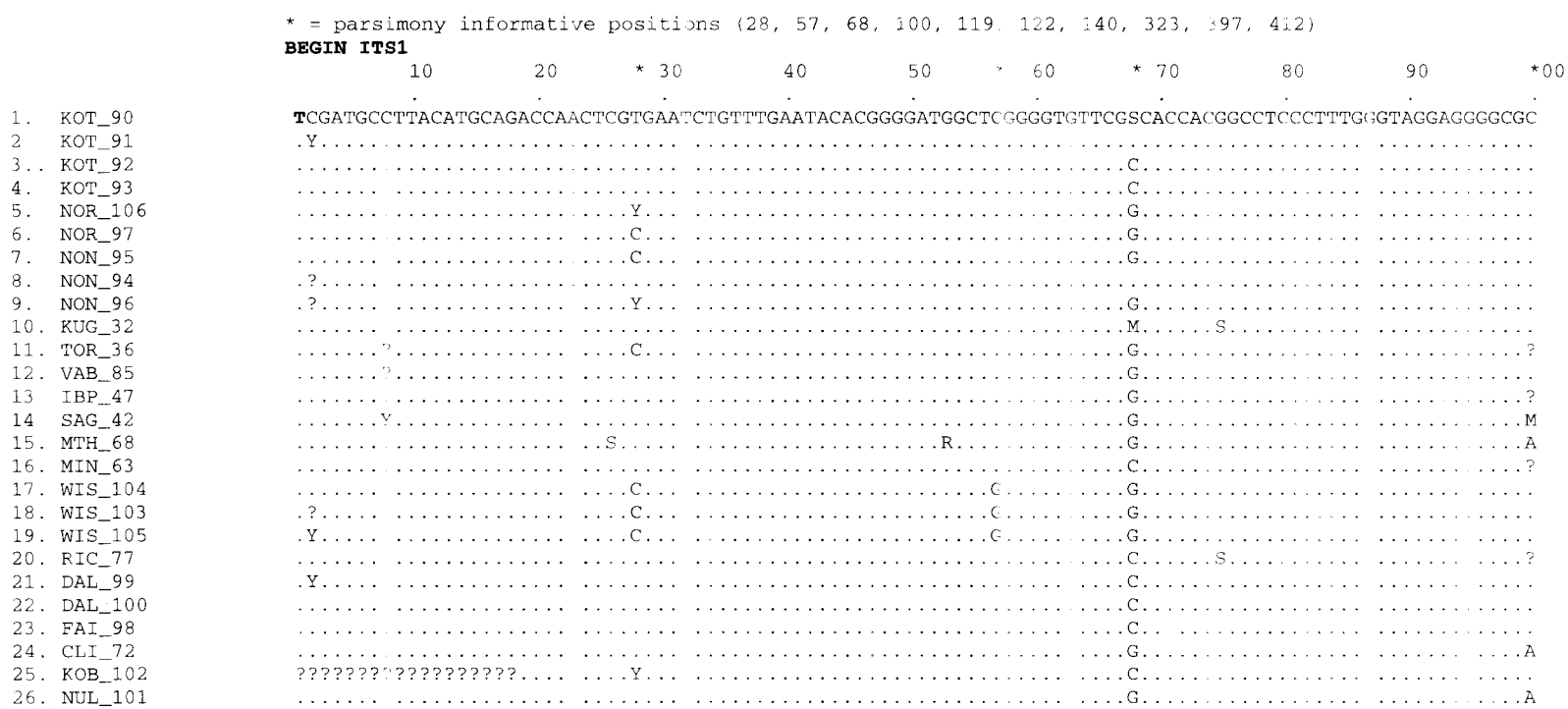


Table 3. continued.

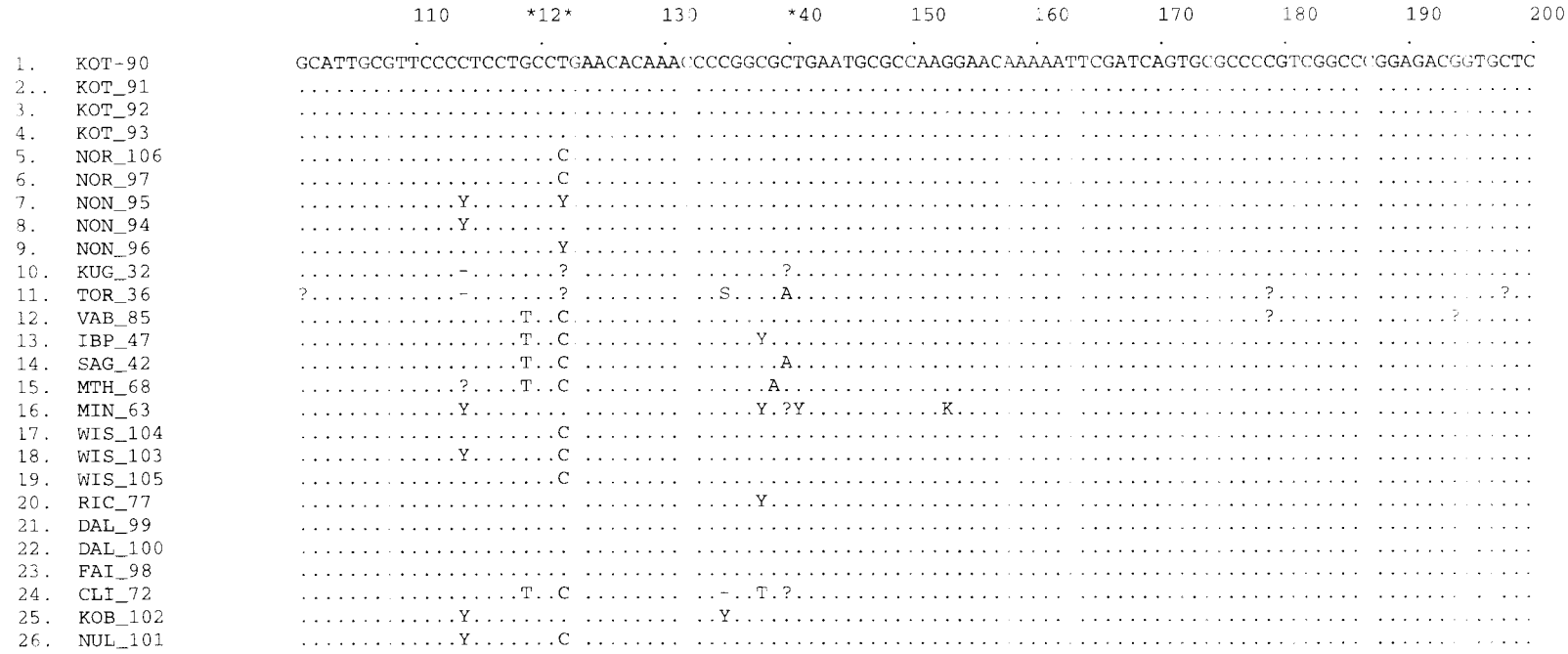


Table 3. continued.

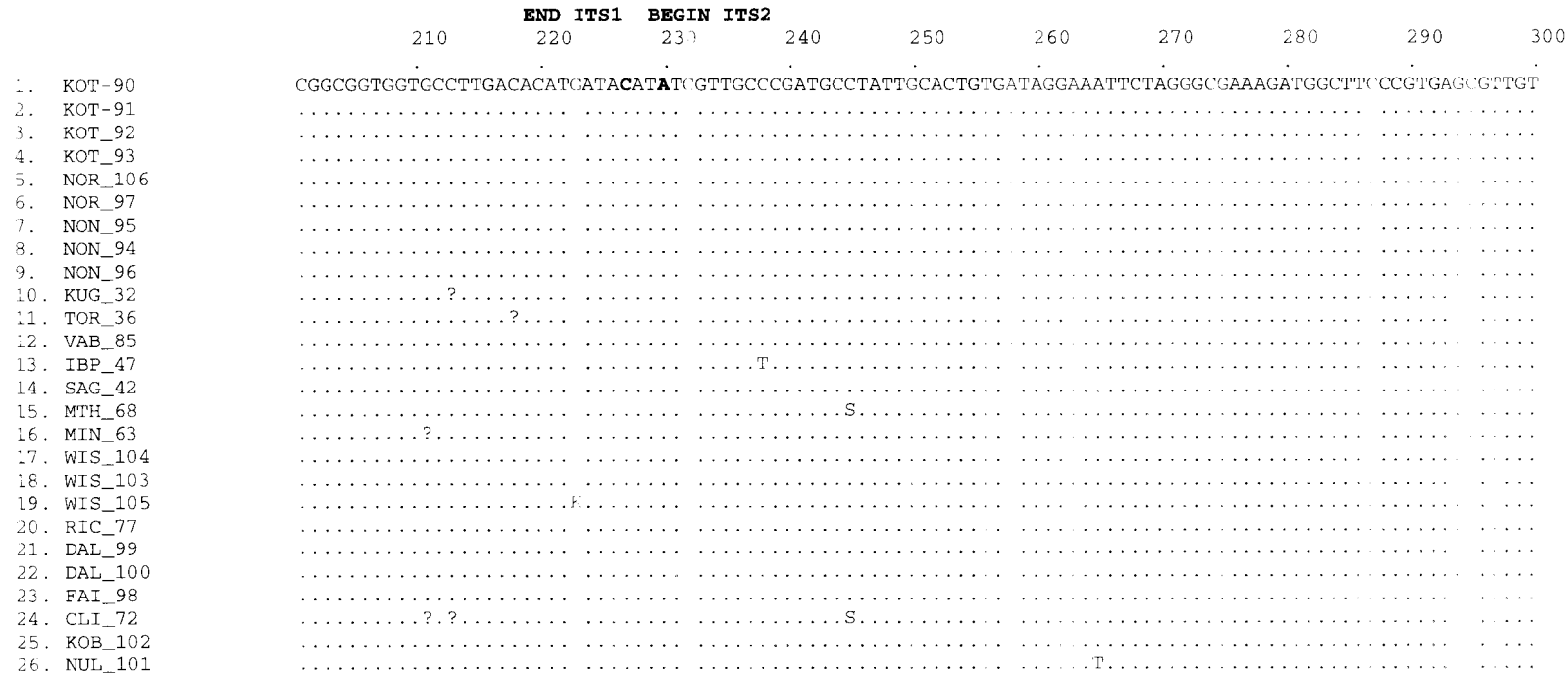


Table 3. continued.

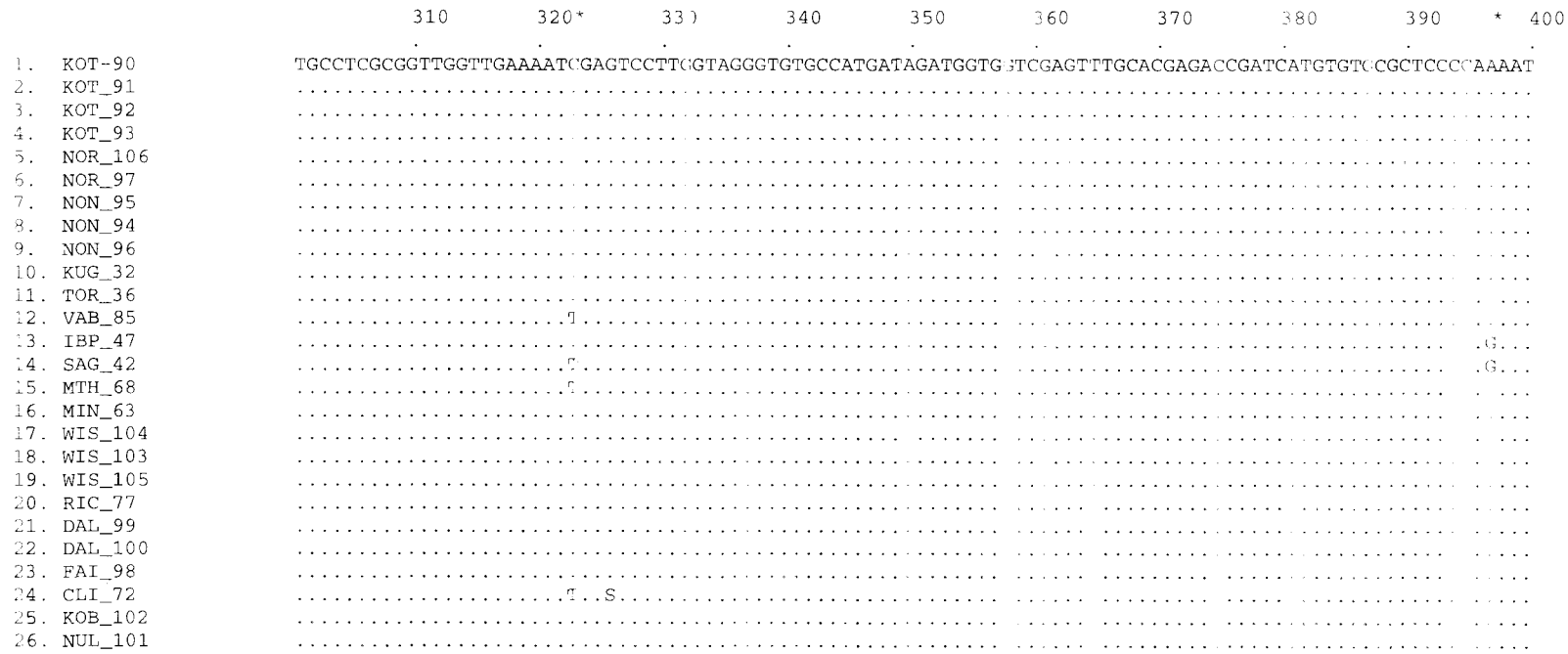


Table 3. continued.

```
                                                                    END ITS2
                                    41*         420         430         440
                                     .           .           .           .
1.   KOT_90          ATGGACTCTTTGACCCCACACGCGTCTTTTGACGCTCAT
2..  KOT_91          ....................................................
3..  KOT_92          ....................................................
4..  KOT_93          ....................................................
5.   NOR_106         ...........T........................................
6.   NOR_97          ...........T........................................
7.   NON_95          ...........T........................................
8.   NON_94          ....................................................
9.   NON_96          ...........K........................................
10.  KUG_32          ....................................................
11.  TOR_36          ...........T................Y.......................
12.  VAB_85          ....................................................
13.  IBP_47          ....................................................
14.  SAG_42          ....................................................
15.  MTH_68          ............................Y.......................
16.  MIN_63          ....................................................
17.  WIS_104         ....................................................
18.  WIS_103         ....................................................
19.  WIS_105         ....................................................
20.  RIC_77          ....................................................
21.  DAL_99          ....................................................
22.  DAL_100         ....................................................
23.  FAI_98          ....................................................
24.  CLI_72          ....................................................
25.  KOB_102         ....................................................
26.  NUL_101         ....................................................
```

Table 4: Jukes-Cantor distances (gamma distribution with shape parameter = 0.006).

Jukes-Cantor distance matrix

	1	2	3	4	5	6	7	8
1 *barnebyana* KOT90	-							
2 *barnebyana* KOT91	0.00000							
3 *barnebyana* KOT92	0.00000	0.00000	-					
4 *barnebyana* KOT93	0.00000	0.00000	0.00000	-				
5 *barnebyana* NOR106	0.00459	0.00461	0.00689	0.00689	-			
6 *barnebyana* NOR97	0.00687	0.00690	0.00917	0.00917	0.00000	-		
7 *barnebyana* NON95	0.00459	0.00461	0.00689	0.00689	0.00000	0.00000	-	
8 *barnebyana* NON94	0.00000	0.00000	0.00000	0.00000	0.00463	0.00694	0.00462	-
9 *barnebyana* NON96	0.00000	0.00000	0.00230	0.00230	0.00000	0.00000	0.00000	0.00000
10 *barnebyana* KUG32	0.00000	0.00000	0.00000	0.00000	0.00457	0.00694	0.00695	0.00000
11 *arctica* TOR36	0.00705	0.00707	0.00950	0.00950	0.00243	0.00242	0.00240	0.00707
12 *barnebyana* VAB85	0.00692	0.00694	0.00926	0.00926	0.00694	0.00926	0.00927	0.00696
13 *arctica* IBP47	0.00921	0.00923	0.01154	0.01154	0.00923	0.01156	0.01156	0.00925
14 *arctica* SAG42	0.01152	0.01154	0.01383	0.01383	0.01154	0.01385	0.01385	0.01156
15 *jordalii* MTH68	0.01156	0.01158	0.01394	0.01394	0.01156	0.01387	0.01385	0.01157
16 *koyukukensis* MIN63	0.00000	0.00000	0.00000	0.00000	0.00699	0.00934	0.00698	0.00000
17 *koyukukensis* WIS104	0.00688	0.00692	0.00917	0.00917	0.00457	0.00457	0.00457	0.00696
18 *koyukukensis* WIS103	0.00690	0.00691	0.00921	0.00921	0.00459	0.00459	0.00459	0.00692
19 *koyukukensis* WIS105	0.00688	0.00690	0.00917	0.00917	0.00457	0.00457	0.00457	0.00694
20 *gracilis* RIC77	0.00000	0.00000	0.00000	0.00000	0.00694	0.00926	0.00695	0.00000
21 *gracilis* DAL99	0.00000	0.00000	0.00000	0.00000	0.00691	0.00921	0.00691	0.00000
22 *gracilis* DAL100	0.00000	0.00000	0.00000	0.00000	0.00689	0.00917	0.00689	0.00000
23 *gracilis* FAI98	0.00000	0.00000	0.00000	0.00000	0.00689	0.00917	0.00689	0.00000
24 *tananensis* CLI72	0.01168	0.01170	0.01407	0.01407	0.01172	0.01405	0.01406	0.01172
25 *kobukensis* KOB102	0.00000	0.00000	0.00000	0.00000	0.00721	0.00722	0.00476	0.00000
26 *nigrescens* NUL101	0.00687	0.00688	0.00917	0.00917	0.00687	0.00919	0.00918	0.00690

Jukes-Cantor distance matrix (continued)

	9	10	11	12	13	14	15	16
9 *barnebyana* NON96	-							
10 *barnebyana* KUG32	0.00228	-						
11 *arctica* TOR36	0.00242	0.00696	-					
12 *barnebyana* VAB85	0.00465	0.00692	0.01172	-				
13 *arctica* IBP47	0.00693	0.00921	0.01408	0.00696	-			
14 *arctica* SAG42	0.00923	0.00919	0.01159	0.00461	0.00693	-		
15 *jordalii* MTH68	0.00925	0.01152	0.01402	0.00461	0.00932	0.00690	-	
16 *koyukukensis* MIN63	0.00232	0.00000	0.00711	0.00939	0.01162	0.01161	0.01170	-
17 *koyukukensis* WIS104	0.00228	0.00696	0.00705	0.00928	0.01156	0.01385	0.01387	0.00936
18 *koyukukensis* WIS103	0.00229	0.00697	0.00706	0.00930	0.01158	0.01388	0.01386	0.00939
19 *koyukukensis* WIS105	0.00228	0.00696	0.00705	0.00928	0.01156	0.01385	0.01387	0.00937
20 *gracilis* RIC77	0.00232	0.00000	0.00952	0.00932	0.01154	0.01388	0.01166	0.00000
21 *gracilis* DAL99	0.00229	0.00000	0.00952	0.00929	0.01156	0.01385	0.01396	0.00000
22 *gracilis* DAL100	0.00230	0.00000	0.00950	0.00926	0.01154	0.01383	0.01394	0.00000
23 *gracilis* FAI98	0.00230	0.00000	0.00950	0.00926	0.01154	0.01383	0.01394	0.00000
24 *tananensis* CLI72	0.00941	0.01161	0.01168	0.00469	0.00699	0.00463	0.00467	0.00938
25 *kobukensis* KOB102	0.00236	0.00000	0.00742	0.00971	0.01209	0.01449	0.01460	0.00000
26 *nigrescens* NUL101	0.00457	0.00688	0.00934	0.00923	0.00921	0.01150	0.00921	0.00693

Table 4. continued.

Jukes-Cantor distance matrix (continued)

	17	18	19	20	21	22	23	24
17 *koyukukensis* WIS104	-							
18 *koyukukensis* WIS103	0.00000							
19 *koyukukensis* WIS105	0.00000	0.00000	-					
20 *gracilis* RIC77	0.00928	0.00932	0.00928	-				
21 *gracilis* DAL99	0.00921	0.00921	0.00919	0.00000	-			
22 *gracilis* DAL100	0.00917	0.00921	0.00917	0.00000	0.00000	-		
23 *gracilis* FAI98	0.00917	0.00921	0.00917	0.00000	0.00000	0.00000	-	
24 *tananensis* CLI72	0.01409	0.01412	0.01409	0.00937	0.01410	0.01407	0.01407	-
25 *kobukensis* KOB102	0.00718	0.00716	0.00717	0.00000	0.00000	0.00000	0.00000	0.01477
26 *nigrescens* NUL101	0.00919	0.00920	0.00919	0.00690	0.00919	0.00917	0.00917	0.00931

Jukes-Cantor distance matrix (continued)

	25	26
25 *kobukensis* KOB102	-	
26 *nigrescens* NUL101	0.00960	-

Table 5. RAPD phenotypes for 89 plants generated from 23 polymorphic markers.

	A	A	A	C	C	C	C	C	D	D	D	D	D	D	D	D	D	D	D	D	D	D
	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	1	1	5	5	5	5	5	3	3	3	3	3	3	7	7	7	7	7	8	8	8
	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0
	3	3	5	5	5	6	7	8	6	6	7	8	9	9	4	4	5	6	7	6	6	9
	0	7	8	9	7	9	0	5	9	9	0	9	0	1	9	9	6	9	0	7	8	9	4
	0	5	4	0	0	0	0	0	0	5	0	9	0	0	0	5	4	5	0	5	0	5	0
KOT-01	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	1	1
KOT-02	1	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	0	1	0	1	0	1	1
KOT-03	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	1	0	1	0	1	1
KOT-04	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	1
KOT-05	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
KOT-06	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0	1	1
KOT-07	1	0	0	1	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
KOT-08	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
KOT-09	?	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
KOT-10	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
NOR-11	?	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	1
NOR-12	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	1
NOR-13	1	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	1	1
NOR-14	1	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0
NOR-15	1	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1
NOR-16	1	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	1
NOR-17	1	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1
NOR-18	1	0	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	1
NOR-19	1	0	0	1	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	1	1	0	1
NOR-20	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	1
NON-21	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0	?	1
NON-22	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	1
NON-23	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
NON-24	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	1
NON-25	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
NON-26	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
NON-27	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
NON-28	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
NON-29	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
NON-30	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
KUG-31	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1
KUG-32	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	1	0	1
KUG-33	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	1
KUG-34	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	1
KUG-35	1	0	0	?	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1
TOR-36	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1
TOR-37	1	0	0	1	0	1	0	0	0	0	0	?	0	0	1	0	0	0	0	0	0	1	1
TOR-38	1	0	0	1	1	1	0	0	0	1	0	?	0	0	1	1	0	0	0	0	0	1	1
TOR-39	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	1	1
TOR-40	1	0	0	1	0	1	0	0	0	1	0	?	0	0	1	1	1	0	0	0	0	0	1
SAG-41	0	1	1	1	0	1	0	0	1	1	1	0	0	0	1	1	?	0	1	0	1	0	0
SAG-42	0	1	1	1	0	1	0	0	1	1	1	0	0	0	1	1	?	0	1	0	0	0	0
SAG-43	0	1	1	1	0	1	0	0	1	1	1	0	0	0	1	1	1	1	1	0	0	0	0
SAG-44	0	0	1	1	0	1	0	0	1	1	1	0	0	0	1	1	1	0	1	0	0	0	0
SAG-45	0	0	1	1	0	1	0	0	1	1	1	0	0	0	1	0	0	1	1	0	0	0	0
IBP-46	0	0	1	0	0	1	0	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0
IBP-47	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	0
IBP-48	?	0	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0
IBP-49	?	0	1	0	0	1	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0
IBP-50	0	0	1	0	0	1	1	0	1	1	0	0	0	0	0	1	0	0	?	0	0	0	0
PEA-51	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1
VIC-52	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1

Table 5. continued.

	A 1 1 / 1 3 0 0	A 1 1 / 1 3 7 5	A 1 1 / 1 5 8 4	C 0 5 / 1 5 9 0	C 1 5 / 0 5 7 0	C 1 5 / 0 6 9 0	C 1 5 / 0 7 0 0	C 1 5 / 0 8 5 0	D 0 3 / 0 6 9 0	D 0 3 / 1 6 9 5	D 0 3 / 1 7 0 0	D 0 3 / 1 8 9 0	D 0 3 / 1 9 0 0	D 0 3 / 1 9 1 0	D 0 7 / 0 4 9 0	D 0 7 / 0 4 9 5	D 0 7 / 0 5 6 4	D 0 7 / 0 6 9 5	D 0 7 / 0 7 0 0	D 0 8 / 0 6 7 5	D 0 8 / 0 6 8 0	D 0 8 / 0 6 9 5	D 0 8 / 0 9 4 0
WIS-53	1	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1
WIS-54	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1
WIS-55	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1
WIS-56	?	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
WIS-57	1	0	0	1	0	1	0	0	?	1	0	0	0	0	1	0	0	0	0	0	1	1	1
WIS-58	1	0	0	1	0	1	0	0	?	1	0	0	0	0	1	0	0	0	0	0	0	1	1
WIS-59	?	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
WIS-60	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
WIS-61	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1
MIN-62	0	1	1	1	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
MIN-63	0	0	1	1	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
MIN-64	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
MIN-65	0	0	1	1	0	1	0	1	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0
MIN-66	0	1	1	1	0	0	0	1	0	1	1	0	0	0	1	1	0	0	0	0	1	0	0
MTH-67	1	0	0	0	1	1	0	0	0	0	0	1	1	0	?	?	0	0	0	?	1	0	1
MTH-68	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
MTH-69	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
MTH-70	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1
MTH-71	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	0	1
CLI-72	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0
CLI-73	0	0	1	0	0	0	1	0	0	0	0	1	1	0	1	1	0	0	1	1	0	0	0
CLI-74	0	0	1	0	0	1	1	0	0	0	0	1	1	0	?	?	0	0	1	0	1	0	0
CLI-75	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
CLI-76	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
RIC-77	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1	0	0
BIR-78	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1
BIR-79	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1
RIC-80	1	0	1	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0
RIC-81	1	0	?	0	0	1	1	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	1
RIC-82	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1
RIC-83	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	1
BIR-84	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	1
VAB-85	0	0	0	0	0	1	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0
VAB-86	0	0	1	0	0	1	0	0	1	1	1	0	0	0	1	0	0	0	1	1	0	0	0
VAB-87	0	0	1	0	0	1	0	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0
VAB-88	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0
VAB-89	0	0	1	0	0	1	0	0	1	1	1	0	0	0	1	0	0	0	1	1	0	0	0

Appendix 1. Table of morphological variation in *O. arctica* and *O. campestris* complexes in Alaska. This table was compiled from the following morphological sources: Barneby (1952), Cody (1996), Elisens and Packerd (1980), Hulten (1968), Porsild (1951), Porsild and Cody (1980), Welsh (1967, 1968, 1974, 1991), Wiggins and Thomas (1962), Yurtsev (1993, 1999)).

	plants/habit	flowers per raceme	inflorescence	scape	flower size, orient., color	corolla color	calyx tube	calyx teeth	stipules
O. arctica var. *arctica*	compact habit mostly less than 15 (22) cm tall less than 5 cm high	rarely more than 5, (2) 5 (10), (9), 2-5 (8)	sub-capitate, not elongate in fruit	6-21cm, (6) 8-10 cm high, erect	large and showy, (14)16-22 mm, >1.5-2.5 cm long	pink, bluish, dark purple, rarely white	calyx 0.8-1.2 cm, tube 5-8.7mm, subulate-tiangular, deltoid to linear-lanceolate, dark and light pilose (villose)	1.5-6mm, 1/2 as long as tube, linear lanceolate teeth	10-20 mm long, connate, densely pilose, base shaggy with white or yellow hairs, papery, free part white or yellow, glabrous or hirsute, clavate processes
O. arctica var. *barnebyana*	mostly less than 15 (22) cm tall	2-8 (9)	subcapitate	9-15 (20) cm long	(14)16-22 mm	white fading cream, keel tip maculate	linear, triangular, villous with light and dark hairs		adnate to petioles, 10-25 mm long, free end acuminate, 7-12mm long, pilose (dorsally) to glabrate in age, ciliate and beset with marginal processes
O. arctica var. *koyukukensis*	mostly more than 15 cm tall to 3 dm.	9-16 (many)	subcapitate, elongate in age		20 mm, spreading at anthesis	purple-blue	9-11mm long, 3--4 mm,	subulate, 3-4mm	over 2 cm, gray, yellow or straw to black, pilose (dorsally) to glabrate in age
O. campestris ssp. *gracilis*	taller species, mostly greater than 15 cm tall	6-25, 10 to many flowered	capitate young, elongate in age	3.5-30 cm long	10-17 mm long	whitish,yellowish, or purple tinged, keel tip maculate or immaculate	black and white hirsute, 4--8, 1--3 mm, tirangular to linear-subulate	subulate	papery white, pale yellow (straw) to black, pilose becoming glabrate, clavate processes, ciliae
O. campestris ssp. *jordalii*	taller species, 12-20 cm tall	3-7 (>10), usually less than 10 flowered	subcapitate, oblong, scarcely elongate in fruit	2-14 cm long, dark purplish, sparsely sericeous.	10-12mm (16)long,	pale greenish yellow, lemon-yellow, drying ivory-white, sometimes bluish yellow, keel purple	4-5mm, gray-pubescent, triangular to linear subulate	1.5mm, teeth subulate, 1/3 as long as the tube	papery, white, translucent, straw, strigose, clavate processes, blackened, marcescent petioles and stipules
O. tananensis	11-25 (40)	(8) 12-16	capitate, to short eliptic +/- dense elongate fruit		13-15mm long	greenish yellow macula light lilac	5-6mm long, tubular companulate, 1-2mm		white memberanaceous, appressed white pilose
O. kobukensis	to 17 (30) cm	3-6 (8)		7-11 cm	16-18mm	purplish, fading blue	purplish, strigulose, light and dark hairs, short-cylindrical to campanulate, 6mm long	1.5-2 mm, linear lanceolate 1/3 long as tube	persistent, rigid, purplish, margins scarious, ciliate, +/- clavate processes, pilose dorsally, purple, firm

Appendix 1. continued.

	leaves	leaflets	leaflet length	leaflet width	leaflet hairs
O. arctica var. *arctica*	4-7 cm long,1. 5-9 (21) cm, 11-21	ovate, narrowly oblong, lanceolate, oblanceolate, elliptic; some faciculate, villous	9-10mm , (2) 4--10 (40) mm	2-5, 3 mm wide	pilose above and below, simple hairs, white villose beneath, glabrate above
O. arctica var. *barnebyana*	6-15 cm, 4-21 cm	obtuse to acute, somewhat pilose on both surfaces. broadly elliptic to lance-elliptic	4-15 (40) mm long	2-5 mm	somewhat pilose on both surfaces
O. arctica var. *koyukukensis*	4-21 cm, 15-25 foliate	pseudofasciculate, numerous	12--35 mm		
O. campestris ssp. *gracilis*	15-35, (2) 3-13 (30) cm, 9-35 (45)	lance-elliptic, oblong, narrowly lanceolate; some fasciculate, pilose both sides	3--40 mm		pilose on both sides, silky pilose or glabrescent
O. campestris ssp. *jordalii*	4-6 cm, 6-10 cm	narrowly lanceolate, broadly elliptic-oblong to lance-elliptic	3--40 mm		glabrous above, appressed silvery-pubescent beneath
O. tananensis	7-17 (27) cm	faciculate, linear lanceolate, lancolate, oblong elliptic	5-11 (20)mm		
O. kobukensis	(3) 6-10cm long, 13-17 foliate	lanceolate to lance oblong, pilose to glabrate above, strigose beneath	6-16mm	2-3.5 mm wide, rounded at base	strigose to pilose below apex, pilose to glabrate above, margins involute

leaflet number., orient	legumes	ecology/ habitat/distribution	chromosome number(2n)
9-13, (9--) 11--13 (--21) opposite, scattered, pseudoverticillate	10-25 mm, olive green, pubescent, short appressed black + long white spreading hairs, ovoid-acuminate, erect or spreading, up to 3 cm inc. long beak, nearly straight	dry open tundra widely distributed	48 Murray and Kelso, 96 Love and Love (1975)
9--15 opp. or alt., oblong to lanceolate, not fasciculate		narrow endemic	unknown
alternate, opposite or sometimes subverticillte	short, black-hirsute, 14-20 mm long, the beak 5-7 mm long, slightly curved, grayish pubescent, long beak, seeds yellow brown, irregularly reniform, diameter +- 2 mm.	sand bars, tundra meadows endemic, range imperfectly known	48 Love and Love (1975)
11-35, 19-45, opp. or vert/fascic.	1.5-2 cm long including recurved beak, yellow green with mixed black and white hairs, erect sessile, pilose	gravel river banks, sandy terraces, and open slopes widespread	16, 32, 96 Dawe and Murray (1981)
9--11 (--17) opp. or alt. or scattered	12-18 mm long including 5mm beak, sessile within the calyx, greenish under a dense but short and black indument, abruptly beaked	alpine and ridges and meadows, dry tundra, limestone endemic to mts. Of northern Alaska and Y.T.barrens	32 Dawe and Murray (1981)
6-11 (14) verticillate	ovoid-ovoid oblong, pilose, white sericious, admixture of black hairs, abruptly narrowed	steep south facing bluffs, endemic, interior alaska	unknown
13-17, not fascic.	immature legume 17 mm long including style, black pilose, bilocular	sand dunes, narrow endemic, Kobuk River Sand dunes	80 (Murray and Kelso, 1997

Appendix 2. Catalog numbers for *Oxtropis* populations. All vouchers deposited in the University of Alaska Herbarium (ALA). I=ITS, R=RAPD , F=frozen leaf tissue, S=silica gel, L=liquid nitrogen, * = no voucher for that particular specimen, E#=extraction number, AFTC=Alaska Frozen Tissue Collection #.

Population:Collector:Date DNA#	R/I	COLLECTION#	F/S/L	E#	ALA Voucher	AFTC	GenBank ITS1 ITS2
KOT:Moran: July 14 & 24, 1997							
01	R	ADOTS #12	F	177	*	43401	
02	R	OAB-8 #08	F	178	*	43402	
03	R	ADOT-N #14	F	186	*	43403	
04	R	OAB-1 #17	F	187	*	43404	
05	R	OWA-S #15	F	188	*	43405	
06	R	ADOT-S #07	F	215	*	43406	
07	R	OAB-8 #12	F	216	*	43407	
08	R	OWA-N #19	F	217	*	43408	
09	R	OWA-S #25	F	218	*	43409	
10	R	ADOT-N #15	F	219	*	43410	
90	I	OAB-8 #11	F	27	*	43411	AF366298 AF366300
91	I	ADOT-C	F	36	*	43412	AF366302 AF366303
92	I	ADOT-N	F	35	*	43413	AF366304 AF366305
93	I	OAB-1	F	48	*	43414	AF366306 AF366307
		VM97 4-2	F		V129414		
		VM97 2-1	F		V129415		
		VM97 1-1	F		V129416		
		VM97 11-1	F		V129417		
		VM97 7-1	F		V129418		
		VM97 5-1	F		V129419		
NOR:Kerr, Klock, Jorgensen & Moran: July 30, 1997							
11	R	ML #03	F	184	*	43415	
12	R	ML #05	F	185	*	43416	
13	R	CLIFF #06	F	189	*	43417	
14	R	CLIFF #01	F	190	*	43418	
15	R	CLIFF #04	F	191	*	43419	
16	R	ML #19	F	225	*	43420	
17	R	ML #02	F	226	*	43421	
18	R	CLIFF #21	F	227	*	43422	
19	R	ML #25	F	228	*	43423	
20	R	CLIFF#07	F	235	*	43424	
106	I	CLIFF #?	F	10	*	43425	AF366308 AF366309
97	I	CLIFF #?	F	11	*	43426	AF366310 AF366311
		JJ97 10-1	F		V129412		
		JJ97 10-2	F		V129413		

Appendix 2. continued.

NON:Kerr, Klock, Jorgensen & Moran:July 28, 1997							
21	R	NN3 FUNK #06	F	179	*	43427	
22	R	NN6 #08	F	180	*	43428	
23	R	NN7 #16	F	181	*	43429	
24	R	NN6 #15	F	182	*	43430	
25	R	NNR #14	F	183	*	43431	
26	R	NN3 FUNKY #10	F	220	*	43432	
27	R	NN4 #05	F	221	*	43433	
28	R	NN5 #02	F	222	*	43434	
29	R	NN6 #10	F	223	*	43435	
30	R	NN7 #12	F	224	*	43436	
94	I	NN4	F	43	*	43437	AF366314 AF366315
95	I	NN5	F	15	*	43438	AF366312 AF366313
96	I	NN5-S	F	44	*	43439	AF366316 AF366317
		JJ97 11-1	F		V129401	43440	
		JJ97 11-2	F		V129402	43441	
		JJ97 11-3	F		V129403	43442	
		JJ97 11-4	F		V129404	43443	
		JJ97 12-1	F		V129405	43444	
		JJ97 13-1	F		V129406	43445	
		JJ97 13-2	F		V129407	43446	
		JJ97 15-1	F		V129408	43447	
		JJ97 16-1	F		V129409	43448	
		JJ97 16-2	F		V129410	43449	
		JJ97 16-3	F		V129411	43450	
KUG:Jorgensen & Suring:July 4,1999							
31	R	JJ99 3-1	S	126	V129382	43451	
32	R/I	JJ99 3-2	S	136	V129383	43452	AF366318 AF366319
33		JJ99 3-5	S	157	*	43453	
35	R	JJ99 3-10	S	148	V129384	43454	
34	R	JJ99 3-13	S	158	V129385	43455	
TOR:Jorgensen & Suring:July 6, 1999							
		JJ99 4-6	S		V129386	43456	
36	R/I	JJ99 4-7	S	137	V129387	43457	AF366320 AF366321
37	R	JJ99 4-9	S	144	V129388	43458	
38	R	JJ99 4-15	S	149	V129389	43459	
39	R	JJ99 4-11	S	159	*	43460	
40	R	JJ99 4-21	S	160	*	43461	
		JJ99 4-18	S		V129390	43462	
		JJ99 4-24	S		V129391	43463	

Appendix 2. continued.

IBP:Batten & Jorgensen: July 13, 1999							
46	R	JJ99 8-1	S	130	V129367	43464	
47	R/I	JJ99 8-2	S	140	V129368	43465	AF366324 AF366325
		JJ99 8-3	S		V129369	43466	
		JJ99 8-4	S		V129370	43467	
		JJ99 8-5	S		V129371	43468	
49	R	JJ99 8-6	S	170	V129372	43469	
48	R	JJ99 8-11	S	152	V129373	43470	
50	R	JJ99 8-9	S	171	*	43471	
SAG:Batten & Jorgensen: July 12, 1999							
41	R	JJ99 5-1	S	129	V129355	43472	
42	R/I	JJ99 5-2	S	138	V129356	43473	AF366326 AF366327
43	R	JJ99 5-4	S	150	*	43474	
44	R	JJ99 5-8	S	161	V129362	43475	
45	R	JJ99 5-15	S	162	*	43476	
VAB:Batten & Jorgensen: July 12, 1999							
87	R	JJ99 6-1	S	145	V129349	43477	
85	R/I	JJ99 6-2	S	139	V129350	43478	AF366322 AF366323
		JJ99 6-3	S		V129351	43479	
86	R	JJ99 6-4	S	151	V129352	43480	
		JJ99 6-5	S		V129353	43481	
		JJ99 6-6	S		V129354	43482	
88	R	JJ99 6-15	S	163	*	43483	
89	R	JJ99 6-20	S	164	*	43484	
MTH:Batten & Jorgensen:July 15, 1999							
67	R	JJ99 10-1	S	132	V129336	43485	
68	R/I	JJ99 10-2	S	141	V129337	43486	AF366299 AF366301
		JJ99 10-4	S		V129338	43487	
		JJ99 10-5	S		V129339	43488	
69	R	JJ99 10-6	S	153	V129340	43489	
		JJ99 10-7	S		V129341	43490	
		JJ99 10-9	S		V129342	43491	
70	R	JJ99 10-10	S	166	V129343	43492	
71	R	JJ99 10-11	S	167	V129344	43493	
		JJ99 10-13	S		V129345	43494	
		JJ99 10-14	S		V129346	43495	
		JJ99 10-17	S		V129347	43496	
		JJ99 10-19	S		V129348	43497	

Appendix 2. continued.

MIN:Batten & Jorgensen:July 16, 1999							
63	R/I	JJ99 11-2	S	142	V129374	43498	AF366328 AF366329
64	R	JJ99 11-5	S	154	V129376	43499	
		JJ99 11-6	S		V129377	43500	
65	R	JJ99 11-7	S	168	V129378	43501	
66	R	JJ99 11-8	S	169	*	43502	
		JJ99 11-9	S		V129379	43503	
		JJ99 11-11	S		V129380	43504	
		JJ99 11-12	S		V129381	43505	
62	R	JJ99 11-1	S	133	V131908	43506	
WIS:Batten & Jorgensen: June 22 & Aug, 8, 1998							
53	R	JJ98 12-6	L	196	*	43507	
54	R	JJ98 12-5	L	198	*	43508	
55	R	JJ98 12-3	L	199	*	43509	
56	R	JJ98 12-2	L	200	*	43510	
57	R	JJ98 12-4	L	201	*	43511	
59	R	JJ98 #3	L	212	V131913	43512	
58	R	JJ98 #5	L	211	V131914	43513	
60	R	JJ98 #1	L	213	*	43514	
61	R	JJ98 #2	L	214	*	43515	
103	I	V. #4 6/22/98	L	24	V	43516	AF366332 AF366333
104	I	V. #3 6/22/98	L	25	*	43517	AF366330 AF366331
105	I	6/22/98	L	33	*	43518	AF366334 AF366335
		6/22/98#10			V131915	43519	
		6/22/98#11			V131916	43520	
		6/22/98#12			V131917	43521	
		6/22/98#13			V131918	43522	
RIC:Jorgensen June 17, 1999							
		JJ99 1-2	S		V129392	43523	
77	R/I	JJ99 1-8	S	134	V129393	43524	AF366336 AF366337
80	R	JJ99 1-8	S	205	V129393	43525	
81	R	JJ99 1-15	S	206	V131909	43526	
82	R	JJ99 1-10	S	207	*	43527	
83	R	JJ99 1-4	S	208	*	43528	
BIR:Jorgensen:1999							
78	R	JJ99 12-1	S	146	*	43529	
79	R	JJ99 12-3	S	176	*	43530	
84	R	JJ99 12-5	S	209	*	43531	

Appendix 2. continued.

CLI:Jorgensen: July 1 & July 17, 1999							
		JJ99 1-21	S		V129395	43532	
75	R	JJ99 2-1	S	155	V129396	43533	
		JJ99 2-2	S		V129397	43534	
		JJ99 2-3	S		V129398	43535	
74	R	JJ99 2-4	S	147	V129399	43536	
72	R/I	JJ99 2-5	S	135	V129400	43537	AF366344 AF366345
73	R	JJ99 2-6	S	143	*	43538	
76	R	JJ99 2-8	S	156	*	43539	
KOB:Parker:Aug. 20, 1997							
102	I	7689CP	S	18	V123557	43540	AF366346 AF366347
DAL:Batten & Jorgensen:June 22, 1998							
100	I	V. #2 MI 187	S	32	V131910	43541	AF366340 AF366341
99	I	V. #1N of CG	S	26	V131911	43542	AF366338 AF366339
NUL:Parker:July 12,1997							
101	I	7256CP	S	34	V123257	43543	AF366348 AF66349
PEA:Parker: July 28, 1999							
51	R	9115CP	S	202	V127701	43544	
VIC:Parker: July 23, 1999							
52	R	9107CP	S	203	V127693	43545	
FAI:Jorgensen:July 16, 1998							
98	I	Anne's Greenh.	S	37	*	43546	AF366342 AF366343